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A Summary of Current Program 7/1/67

and Preliminary Report of Progress

for 7/1/66 to 6/30/67

ANIMAL DISEASE AND PARASITE

RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

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This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1966, and June 30, 1967. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

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INTRODUCTION

The Animal Disease and Parasite Research Division administers a national program of basic and applied research on diseases of cattle, poultry, swine, sheep, horses, and fur-bearing animals. The Division consists of 3 large laboratories and 11 smaller, specialized laboratories. The large ones are the Beltsville Parasitological Laboratory, Beltsville, Maryland, the National Animal Disease Laboratory, Ames, Iowa, and the Plum Island Animal Disease Laboratory at Greenport, New York. The research at these locations covers, respectively, animal parasites, animal diseases existing in the United States, and foreign animal diseases. The smaller, specialized laboratories are located as follows:

Southeast Poultry Research Laboratory, Athens, Georgia.
Regional Animal Parasite Laboratory, Auburn, Alabama, with
substation at Experiment, Georgia.
Endoparasite Vector Pioneering Research Laboratory, Pullman,
Washington.
Toxicological Research Laboratory, Kerrville, Texas.
Southwestern Veterinary Toxicology and Livestock Insects Research
Laboratory, College Station, Texas.
Ectoparasite Vector Research Laboratory, Denver, Colorado.
Poisonous Plant Research Laboratory, Logan, Utah.
Parasite Research Laboratory, Albuquerque, New Mexico.
Parasite Research Laboratory, Las Cruces, New Mexico.
Swine Parasite Research Laboratory, Tifton, Georgia.
Cooperative Research at the East African Veterinary Research
Organization, Muguga, Kabete, Kenya, East Africa.

The Division also engages in other research involving 44 cooperative projects and research contracts at various universities and State Experiment Stations. Also, research on 22 projects on animal diseases and parasites in 7 foreign countries is sponsored by the Division under Public Law 480. The Division's research program is coordinated by the Office of the Director, located at Beltsville, Maryland.

The Animal Disease and Parasite Research Division has contributed many significant research findings aimed at reducing the heavy losses to the livestock industry resulting from animal diseases. Several of these research discoveries have accounted for savings to the livestock industry in excess of the total cost of animal disease research in the U. S. Department of Agriculture since the inception of the Bureau of Animal Industry in 1887. Among these discoveries are the isolation and description of the genus of bacteria known as Salmonella; the role of arthropod vectors in spreading infectious diseases; the cause of hog cholera and the development of the

first immunization procedure for this disease; the first successful treatment for hookworms in animals and man; the development of strain 19 vaccine to prevent brucellosis, and the discovery of the cause of hyperkeratosis in cattle. Some of the more recent accomplishments by this Division are, in priority order for the first 14 -

The Plum Island Animal Disease Laboratory has rendered assistance to the duck industry on Long Island by confirming a foreign disease lethal to ducks that appeared on Long Island in January 1967. A virus was isolated and proved to be identical to the duck plague virus which is endemic in The Netherlands. Knowledge was developed regarding the disease transmission, susceptibility of ducks at different ages, the immune response, the characteristics of the virus, and the resistance of the virus to heat and disinfectants. This information was most useful and used as guidelines in the development of regulatory measures. In this investigation, conventional and modern methods of disease diagnosis were used.

Agar gel diffusion test used to detect group specific antibody in sheep and cattle infected with bluetongue (BT) disease. A noninfected soluble antigen was prepared from BT-infected cell cultures, and good results were obtained when it was tested using vaccinated sheep and artificially-infected sheep and cattle. A test of this type will be helpful in determining the incidence of BT disease in the sheep and cattle population.

Obtaining nonpathogenic foot-and-mouth disease virus for possible use as a vaccine. A procedure has been investigated which may prove useful for rapidly obtaining a living virus vaccine against foot-and-mouth disease. The procedure consists of growing virulent virus in cultures of dog kidney cells, which are relatively resistant to the virus. After only a few passages in dog cells, the virus changed from a highly infectious agent for cattle to one that produced no lesions characteristic of the disease. Cattle inoculated with this virus became immune to infection with the original virulent virus. Experiments indicate that the decrease in ability to produce disease may be related to production of virus in dog cells that interferes with the infective process.

Horses harbor parasites (become carriers) of equine piroplasmosis after recovery. In work at the Beltsville Parasitological Laboratory, 14 of 30 test horses have remained "carriers" of the agents of piroplasmosis after about 2 years, although the horses no longer had signs of the disease. This fact was determined by using a diagnostic (complement fixation) procedure, and by inoculating blood from the test (donor) horses into susceptible horses. When parasites were present in the donors, the inoculated horses developed the disease, showing that the test horses were "carriers." This research is important to control work, because it shows that horses may not be cured of the disease when signs disappear. Ticks, the vectors,

feeding on such apparently cured horses (carriers) may acquire the parasites and transmit them to other horses, thereby spreading the disease.

Evidence has been accumulated indicating that the protein coat of foot-and-mouth disease virus is built up entirely of a single species of polypeptide molecules of lower molecular weight. This finding permits work to be started on determining which grouping of amino acids in the polypeptide is responsible for the immunizing of infected or vaccinated animals.

New rapid test for bovine anaplasmosis being developed at the Beltsville Parasitological Laboratory. Encouraging results have been obtained in laboratory trials with a new diagnostic test. This test involves mixing, on an especially prepared card, small amounts of blood serum from an animal to be diagnosed and a newly devised agglutinating antigen prepared from the causal parasite, Anaplasma marginale, after its removal from the red blood cells. The test so far has been as effective in differentiating between infected and noninfected animals as is the standard (complement fixation) test. Moreover, it can be completed in about 15 minutes as compared to several hours for the standard method. The new testing procedure offers promise of a ready, economical tool for screen-testing cattle for anaplasmosis under field conditions, a need that has not heretofore been met.

Complement fixation, the most reliable diagnostic test for equine piroplasmosis. In diagnostic work on equine piroplasmosis at the Beltsville Parasitological Laboratory, the complement fixation (CF) test has proved more specific and reliable than either agar gel diffusion or fluorescent antibody procedures. The latter tests require antiserums of higher titer than does the CF test, may not be effective until late in the course of the disease, may have high degrees of nonspecificity, and may fail to differentiate between negative animals and animals that are "carriers." Detection of carrier animals is highly important in control work. To date, the CF test has consistently evaluated the carrier state in experimental animals.

Naturally-occurring substances in milk affect the growth of Streptococcus agalactiae. Growth of Streptococcus agalactiae in milk is partially inhibited by the enzyme lactoperoxidase. Studies of factors that may impart resistance to bovine mastitis showed that the lactoperoxidase system cannot reach its maximum inhibitory activity against this organism because of the presence of an unidentified compound in milk. The compound is more heat stable than lactoperoxidase and can be separated from it by dialysis or ultrafiltration. Isolation and identification of the compound would permit an examination of its relationship to diet and the development of clinical mastitis.

An improved method for the detection of Salmonella organisms during egg shell penetration studies. The most common Salmonella occurring in both man and animal in the United States is Salmonella typhimurium. When tetrathionate

brilliant green enrichment broth is used, contaminants pose a minor problem in studying the penetration pattern of this important Salmonella organism into the shell and shell membranes of chicken eggs. By adding 1:15,000 neotetrazolium chloride to the basal tetrathionate broth, a method for early Salmonella detection has been developed. This method has resulted in a considerable saving of both time and money in the egg penetration studies.

Bovine venereal trichomoniasis, a major reproductive disease, may be curable and eradicable by simple means. A synthetic compound, dimetridazole, has exceptional promise as a systemic treatment. Infected bulls have been cured when dimetridazole was given by capsule or admixed with their feed for 5 successive days, or when administered as a single intravenous injection. Preliminary results of current research indicate that infected cows respond favorably to similar regimens. Thus, it is probable that treatment can be carried out on a herd basis, thereby eliminating the possibility of reinfection and permitting the return of all cattle to production much sooner.

Active immunity by oral administration of killed Pasteurella multocida. Very little has been reported on the stimulation or immunity by oral administration of killed microorganisms. Preliminary studies showed that chickens and turkeys can be immunized by this procedure. Relatively large doses of vaccine were required to induce immunity to fowl cholera by this method as compared with parenteral administration. Three doses were more effective than 1 or 2 doses containing the same total amount of antigen. Very little correlation was observed between the presence of active immunity and the presence of antibodies detectable by serologic tests. Further studies are planned to indicate the relationship between antibodies induced against oral and parenteral administered antigens.

Teratogenic substances in Veratrum californicum, the cause of cyclopian deformities in sheep, have been isolated. Ovine cyclopia induced by ingestion of V. californicum has been produced experimentally by 4 steroidal alkaloids isolated from the plant. The new alkaloid cyclopamine and its glycoside, designated alkaloid X, and the previously isolated alkaloids, jervine and veratrosine, have produced the effect. Structural characterization of the new alkaloids and the study of mechanism of action are underway. Another type of teratogenic effect centered in motor control of the limbs is due to the alkaloid veratramine isolated from the plant.

Vaccination of calves at 2 to 3 months of age effective in brucellosis. In a cooperative experiment with the Ohio Agricultural Research and Development Center, it was established that calves vaccinated at 2 or 3 months of age with strain 19 developed an immunity comparable to that obtained by vaccination at older ages. In addition, vaccination at the younger ages markedly reduced the problem of persistent postvaccinal agglutinin titers.

Early detection of paratuberculous cattle. Cultural examination of fecal specimens have been compared with the complement fixation, gel diffusion precipitation, intradermic johnin tests, and microscopic examination of fecal specimens as methods of early detection of paratuberculous cattle. Cultural examination of fecal specimens was far superior to the other methods. This procedure will be tested as an aid for the control and eventual elimination of the disease in infected herds.

"Overwintering" of bluetongue virus in cattle. In November, a 3-year-old Hereford cow on a farm in northeastern Colorado had signs of a vesicular disease. The cow was chronically infected and by the end of March had lost approximately 600 lbs. In 2 separate attempts, 60 days apart, bluetongue virus was isolated from its blood. This finding strongly indicates a "carrier" state for cattle infected under natural conditions as well as a mechanism for the "overwintering" of an arbor virus.

Morphogenesis and nucleic acid identification in duck virus enteritis (duck plague virus). Examination of thin sections of infected duck embryo cells showed that duck virus enteritis developed in the cell nucleus, migrated to the cytoplasm and then into intercellular spaces. Enzymic digestion revealed a DNA viral core and a protein envelope in the mature particle. These experiments indicated that duck virus enteritis was a newly-described herpes virus. This virus was just recently implicated in a disease outbreak on eastern Long Island duck farms.

Isolation of bluetongue virus from an aborted bovine fetus. Bluetongue virus was isolated from the spleen of an aborted fetus that was estimated to be 6 months old. The dam was a 2-year-old Shorthorn heifer naturally infected with the virus in the early trimester of its gestation under a natural epizootic of the disease. The dam had initial signs of a vesicular disease and subsequently lost her hair and sloughed her hoofs. The heifer had a very prolonged period of recovery. A 2nd known BT-infected and pregnant Shorthorn heifer, involved in the same epizootic, carried a calf to terminal gestation. However, the newborn calf had congenital anomalies. These findings are important in understanding the epizootiology of the disease.

Studies indicate that an agent of equine piroplasmosis multiplies in the tick which transmits it. In work at the Beltsville Parasitological Laboratory to evaluate transmission of an agent (Babesia caballi) of equine piroplasmosis, indications were that the tick is not merely a passive carrier, but serves as a "growth medium" for multiplication of the parasites. In series of ticks fed on infected horses and examined microscopically at intervals thereafter, the numbers of parasites found were many times the probable number ingested by the ticks while feeding. Stages thought to be infective to the horse were in the salivary glands and secretions of ticks in all stages of development. Multiplication of the parasites within the bodies of the vector (tick) would enhance the probability of serious disease in horses preyed upon by infected ticks.

Ticks on cattle lost infections of an agent (*Babesia caballi*) of equine piroplasmosis. In work at the Beltsville Parasitological Laboratory to find ways of controlling piroplasmosis of horses, ticks that acquired infections of one of the agents (*Babesia caballi*) by feeding on horses with the disease, lost the parasites by living for 2 or more generations on cattle. Horses on which these 2nd generation ticks fed remained free of the disease. Other horses exposed to members of the 1st generation of ticks from the cattle acquired piroplasmosis. This finding may offer promise of a ready method of on-pasture control of equine piroplasmosis by pasturing cattle on affected areas in sufficient numbers and long enough for ticks on the areas to develop through 2 generations on the cattle.

Rocky Mountain bighorn sheep, and domestic sheep and cattle have many parasites in common. Specific determinations of the parasites collected from 18 bighorn sheep from 3 separate localities in Montana and of specimens in the Parasite Collection at the Beltsville Parasitological Laboratory, have increased the known number of parasites of bighorn sheep from 34 to 51 species. Seventy per cent of these 51 different species are known parasites of domestic sheep and 35% of cattle. The evidence indicates that there is an interchange of parasites between bighorn sheep, and domestic sheep and cattle. It also indicates that pathogenic worm parasites from domestic sheep were probably responsible for the large losses in flocks of bighorns reported in the early literature soon after domestic sheep were introduced in the western states.

A highly purified ribonuclease inhibitor, dextran sulfate, has been used successfully in the study of foot-and-mouth disease virus replication in tissue culture. This compound has made possible the isolation of ribonuclease-sensitive macromolecules from infected cells, heretofore unobtainable. One of these components, polyribosomes, is now being isolated from both normal and infected cells. Another example is a new high molecular weight RNA-containing component which has properties of an intermediate in the synthesis of 37S foot-and-mouth disease virus RNA. This RNA component is active in a cell-free system and is being studied in great detail.

Lathrogenic substances may be the teratogenic agents in locoweed poisoning. Many of the teratogenic and pathologic effects produced in sheep that ingest the loco plant have been replicated by feeding the sheep certain lathyrogens. Attempted isolation of lathyrogens from loco is now underway.

Resistance of calves to parainfluenza-3 virus respiratory infection (shipping fever). Resistance of calves to apparent parainfluenza-3 virus infection was associated mainly with antibody in the serum, but was influenced by antibody in the nasal secretion. Resistance to inapparent infection of the respiratory mucosa was associated mainly with antibody in the nasal secretion. As sufficient amounts of circulating antibody were formed after either inhala-

tion or intramuscular inoculation of virus, but relatively little (if any) nasal antibody was formed after intramuscular inoculation, these results offer an explanation for failure to establish immunity by conventional methods of vaccination. In addition, they suggest that immunity can be obtained by exposure to aerosols of attenuated parainfluenza-3 virus preparations.

The diagnosis of acute aflatoxicosis in swine. Despite reports of naturally-occurring cases of acute intoxication, little information concerning clinicopathologic effects in the acute condition is available. Young pigs were "fed" quantities of aflatoxin sufficient to produce acute intoxication and death in 24 to 72 hours. A combination of clinicopathologic, pathologic, and chromatographic evidence was considered necessary for diagnosis. Marked alterations were found in liver function and serum levels of certain enzymes (ornithine transcarbamylase and glutamic-oxalacetic transaminase). Metabolites of aflatoxin were found in the urine by using thin layer chromatography.

Diagnosis of psittacoid lamb polyarthrits. The pathological findings of an outbreak of lamb polyarthrits that was caused by a chlamydial agent has been studied. Infected cells from the membrane lining the joint cavity are released into the joint fluid. By centrifuging these cells and rapidly processing and examining by electronmicroscopy, the agent can be demonstrated. An accurate diagnosis of psittacoid polyarthrits can therefore be made in 24 hours. The agent has been used to experimentally produce the disease for additional pathologic investigations.

Infectious bronchitis virus interference with Newcastle disease virus in monolayers of chicken kidney cells. The measurement of infectious bronchitis virus and its antibodies in embryonating eggs can be a lengthy and tedious process. The ability of infectious bronchitis virus to interfere with Newcastle disease virus in chickens and in embryonating eggs was applied to chicken kidney cell cultures. The cell culture technique has been successfully used to measure concentrations of several egg-adapted strains of infectious bronchitis virus and to quantitate the concentration of serum antibodies against this virus. The serologic types of bronchitis virus can be distinguished in as little as 3 days with this technique.

Nodular worm parasite of sheep harmful to calves. The nodular worm, Oesophagostomum columbianum, normally parasitic in sheep, can penetrate the intestinal mucosa of calves and undergo some growth and development in this host. Lesions are produced at the site of penetration. Sexual maturity is not reached and no resistance is stimulated to subsequent infection with O. radiatum, the common nodular worm of cattle. This information contra - indicates the use of management practices that recommend mixed or alternate grazing of pastures by cattle and sheep to control gastrointestinal parasitism.

Larvae of potentially pathogenic gastrointestinal nematode parasites of sheep survive the winter on Mississippi pastures. Infective larvae of 2 stomach worms of sheep, Haemonchus contortus and Ostertagia circumcincta and the eggs of the intestinal threadnecked worm, Nematodirus spathiger, survived the winter months in Mississippi and were capable of infecting lambs grazing the contaminated pastures the next spring. This observation is important in that the survival of these parasites from 1 grazing season to another must be taken into account in planning effective control measures for sheep parasites in this region.

Drug-resistant coccidia may regain sensitivity to coccidiostats. Protozoan drug resistance, unlike that of most bacteria, is relatively stable and persists even when strains are propagated in the absence of the drug. The degree of resistance in 2 experimental strains of avian coccidia was undiminished after 10 passages through unmedicated chickens. One of these strains (resistant to amprolium) was concomitantly passed through chickens fed a low level of acriflavine. After 10 passages, this strain had reverted and was again sensitive to amprolium. These results indicate that drug resistance in coccidia is not irreversible and afford a lead to a possible method of combating this serious and persistent problem of the poultry industry.

New sources of poultry blackhead discovered. Studies at the Beltsville Parasitological Laboratory revealed that field crickets and sowbugs can become sources of infection of poultry with blackhead if birds swallow eggs of the poultry cecal worms that are the vector of the blackhead parasite. Crickets and sowbugs are widespread in nature, occur frequently in premises occupied by poultry, and may be eaten by the poultry. Crickets and sowbugs were artificially exposed to cecal worm eggs known to contain the blackhead parasite. After a time, the crickets and the bugs were fed to turkeys. Infections of cecal worms and blackhead parasites developed in some of the turkeys. Although outward signs of blackhead were not observed in these turkeys, the parasite was present. This work provides new knowledge of ways that poultry may acquire blackhead, a disease that costs the poultry industry several million dollars each year. Moreover, it may serve to identify the source of a costly outbreak of the disease that last year occurred in a drought stricken area where potential sources of infection other than crickets and sowbugs were absent.

Improved developments with the fluorescent micro-plaque assay for hog cholera virus using homologous antiserum for cell culture localization of virus infection sites. Localization of primary virus plaques was accomplished by the incorporation of anti-hog cholera serum into the nutrient medium of infected cell cultures. Plaque size was directly related to incubation time and inversely related to the concentration of antiserum. In the presence of a sufficient concentration of antiserum, the number of plaques did not

increase with continued incubation of infected cultures. In addition, a linear relationship was found between hog cholera virus concentration and plaque counts; titers obtained by plaque assay were essentially the same as 50% cell culture infective dose titers.

Potential mammalian carriers or reservoir hosts of hog cholera virus investigated by in vitro studies of cell susceptibility to virus infection. Twenty-five primary cell cultures, 14 low-passage cell line cultures, and 14 high-passage established cell line cultures, derived from 8 orders and 30 species of mammals, were exposed to virulent and modified hog cholera viruses. Twelve of the 25 primary cell cultures were susceptible to both virulent and modified viruses. Ten of the 14 low-passage cell line cultures were susceptible to virulent virus and 13 of the 14 cultures were susceptible to modified live virus. Seven of the 14 high-passage established cell line cultures were susceptible to virulent virus and 8 of the 14 cultures were susceptible to modified live virus.

Naturally-occurring and experimental pseudocowpox (cattle) lesions from the teat examined by electron microscopy. Teat lesion biopsy and examination provide a rapid and accurate method of diagnosis in 24 hours. The virus did not grow in egg embryos or rabbits, but produced a marked cytopathic effect in primary bovine kidney cell cultures. The latter method provides a convenient method of virus isolation in vitro.

An improved method was developed for obtaining cuticular material from encysted larvae of *Trichinella spiralis*. Nematode cuticle is one of the principal binding sites used to detect the presence of antibody by means of the fluorescent antibody test. One of the problems limiting the use of this test for the diagnosis of trichiniasis in swine has been the difficulty of providing clean cuticular material from encysted trichinae for use as antigen. By holding trichinous pork at 0 F. for 20 days, it has been possible to digest the soft bodies of the encysted larvae together with the meat in which the worms are encysted. When the residue was examined microscopically, about 98% of the larvae were observed to be completely digested and were represented by empty cuticle; the remaining 2% had ghost forms within the cuticle where the larvae had been. By washing and sedimenting this residue, relatively clean, concentrated cuticular antigenic material can be provided for the test.

Existence of discrete strains of *Psoroptes ovis* has been demonstrated. The existence of discrete strains of *Psoroptes ovis*, the common scabies mite of sheep and cattle, has been demonstrated. This observation contributes measurably to understanding the sometimes obscure and evasive epidemiology of psoroptic scabies of sheep and cattle. This concept, furthermore, strongly indicates that tests involving candidate acaricides for the control of scabies should involve only strains of mites of predetermined vigor and high survival potential. Strains differed in degree of pathogenicity to sheep,

transmissibility from sheep to cattle, oversummer survival on sheep, and resistance to contact and systemically active acaricides administered as dips.

Three acaricides found to be effective against Psoroptes ovis. Three organo-phosphorous acaricides (2 phosphorodithioate and 1 dimethyl phosphate compound) are effective for the eradication of the scabies mite, Psoroptes ovis, on sheep and cattle. These broad-spectrum ectoparasitocides are characterized by relatively low toxicity to livestock, and present minimum tissue and milk residue problems. One compound is already registered for use on dairy cattle. Large scale pen tests employing single applications of chemotherapeutic agents have been eminently successful. The drugs may find wide application for use on dairy cattle and goats afflicted with scabies and mange, since no satisfactory agent is currently available for the purpose.

Adult intestinal flukes proved to acquire rickettsial disease. The development of surgical techniques to "short circuit" the adult salmon poisoning disease fluke (Nanophyetus salmincola) cycle has resulted in proof that rickettsia-free flukes can acquire the salmon poisoning disease rickettsia from infected animals, and further, can transmit this acquired infection to healthy animals. This finding confirms the suspicion that an endoparasite can acquire an infectious disease from a definitive host.

Snail vectors of liver flukes in the Southwest. The common liver fluke of sheep and cattle is enzootic in parts of southern Colorado, northern New Mexico, and eastern Arizona. Recent investigations based on natural and experimental infections have shown for the 1st time the identities of snail vectors in these areas. This information will pave the way for an evaluation of methods of controlling the snail vectors through the use of biological and chemical agents.

Age as a factor in immunity to stomach worms of sheep. The capability of lambs to develop immunity to haemonchosis increases with age. This information was obtained by comparing lambs 3 and 6 months old. The procedure was to inoculate the lambs with larvae of an attenuated strain of the parasite from pronghorn antelope, then to remove the immunizing infections with phenothiazine. This step was followed by challenge inoculation with sheep strain Haemonchus larvae to determine the degree of immunity. This information may be used to an advantage by sheep producers by limiting the grazing of younger lambs and consequently their exposure to parasitism.

Surgical implantation permits study of early stages of nematode infections. Guinea pigs have been infected with the nodular worm of cattle via surgical introduction of the infective larvae into isolated portions of their small intestines. The larvae penetrate the intestinal wall in 6-8 hours, and

tissue samples can be obtained easily for study of the processes of penetration and tissue reaction. The nodular worm is one of the most important parasites of cattle, and the study of the crucial early stages of this infection has heretofore been impractical because of the extreme difficulty of locating the sites of initial penetration in the intestines of cattle. This development will enable researchers to use guinea pigs for laboratory studies of infection by nodular worm instead of expensive cattle.

A miniature laser beam device proved to be destructive to oocysts of coccidia. A commercial laser that couples to a microscope was borrowed for the test. The miniature laser beam was projected down through the microscope and the objectives of the microscope, concentrating the beam to less than 10 μ in diameter. The power input was adjustable between 70 and 230 joules of light energy. The resistant oocysts of coccidia of cattle and sheep were disintegrated or, with less power, cracked, so that a dye could penetrate the nearly impervious double outer wall. After 2 weeks, the latter oocysts did not show any evidence of sporulation, indicating that even though the inner contents seemed to be intact, the oocysts had been killed. Additional testing is being conducted, but preliminary evidence suggests that the device can be used for isolating pure cultures by destroying the contaminating species in a mixed culture. This process should prove more effective and faster than the previously used micromanipulation methods of removing unwanted oocysts. It should be effective in isolating pure cultures of nematode larvae also.

Proper ration important for swine infected with Strongyloides ransomi. The small intestinal threadworm of swine, Strongyloides ransomi, is the most important cause of death in suckling pigs in the southeastern area. Experimental studies with weaned pigs have shown that a well balanced and vitamin-fortified ration is very important in helping the pig overcome the deleterious effects of this parasite. Treating the pigs with an anthelmintic for the removal of the parasites is also beneficial, but not as effective as the proper ration. The combination of both treatments was superior to either. The fact that a proper ration is more important than removal of parasites adds valuable new information for use in our management programs.

Radiographic studies show differences in bovine teat canals. Radiographic techniques were utilized to study anatomical features of the teat canal that might be related to native resistance to bovine mastitis. These methods were the first to permit detailed analyses of teat canal dimensions in the living animal. Substantial differences in length and diameter were found between cows and in different glands of the same cow. These differences are being studied relative to their association with ascending infections of the mammary gland.

Observations on parasitic nematodes grown in vitro lead to redescription of life cycles. Based on new morphological characters, development of Stephanurus dentatus and Ascaris lumbricoides, the most damaging roundworms of swine, was restudied and shown to differ at critical points from previously accepted life cycles. Accordingly, the validity of work based on, or correlated with, the latter must now be questioned.

Tetramisole, a new anthelmintic for livestock. Tetramisole, (dl 2,3,5,6-tetrahydro-6-phenylimidazo (2,1-b) thiazole hydrochloride), is effective against adult stages of the large stomach worm, Haemonchus contortus, and the stomach and intestinal hairworms, Trichostrongylus axei and T. colubri-formis, respectively, of sheep and goats. These roundworms interfere with the normal metabolism of the host resulting in morbidity and sometimes death. Preliminary data indicate that tetramisole compares favorably with thiabendazole and purified, micronized phenothiazine as effective treatments against these 3 major parasitic pathogens of ruminants.

Six species of thread-necked strongyles parasitize domestic sheep in the United States. Specific determinations of intestinal thread-necked strongyles in 90 collections of parasites from 21 states revealed that 6 species of Nematodirus parasitize domestic sheep, and not 4 as previously believed. Furthermore, the various species have been confused with one another in publications, and it has been determined that N. abnormalis, previously considered rare, is the second most common species. The 6 species in descending order of their incidence are: N. spathiger, N. abnormalis, N. filicollis, N. lanceolatus, N. helvetianus, and N. davtiani. The last named species was described in Russia, and heretofore was not known to occur in this country. It was found in domestic sheep in Wyoming and in Rocky Mountain bighorn sheep in Montana.

Colostrum (first milk) not necessary for lambs raised artificially. In work at the Beltsville Parasitological Laboratory to find ways of artificially raising lambs parasite-free, colostrum (first milk of the mother) was not essential to growth. Colostrum is generally supposed to be essential to life of the very young animal. Thirty-eight lambs "caught" at the time of birth were raised entirely on an artificial diet. For comparison, 38 others remained 8-24 hours with the mothers to obtain colostrum, and were then fed the artificial diet exclusively. Both groups were raised under rigid sanitation. Both groups remained parasite-free, the growths were comparable, and the number in the 2 groups that died were about the same. The results of this work provide information for a solution to the problem of raising parasite-free lambs. Lambs may safely remain with their mothers for 8-24 hours before isolation and still remain free of parasites.

Two-year-old, parasite-free sheep were as susceptible to infection with large stomach worms as were 2-month-old, parasite-free lambs. Four 2-year-old, parasite-free sheep and eight 2-month-old, parasite-free lambs were infected with 12,000 larvae of the large stomach worm, *Haemonchus contortus*, and later reinfected with 16,000 larvae of the same species of parasite. The hematocrit level of the older sheep stayed within normal limits, whereas that of the younger lambs was depressed. The average worm load of the 2-year-old sheep was 1,800, and that of the lambs, 2,300; however, the range was such that the difference in the final worm loads was insignificant.

The infectivity of foot-and-mouth disease virus RNA is increased by several orders of magnitude by complexing it with the polycation DEAE-dextran. This enhancement will enable researchers to study the infectivity of intracellular replicative forms of viral RNA. It has already led to the isolation of infectious RNA from transmissible gastroenteritis virus of swine.

Nebraska sheep harbor worms capable of causing clinical parasitism under intensive management systems. Sheep on pastures at the U. S. Meat Animal Research Center at Clay Center, Nebraska, harbored small numbers of 4 species of potentially pathogenic gastrointestinal parasites, 2 stomach worms, *Haemonchus contortus* and *Ostertagia circumcincta*, and the intestinal worms, *Trichostrongylus colubriformis*, and *Nematodirus* sp. Future plans for the Center call for greater numbers of sheep and more intensified grazing on irrigated pastures. The increased moisture will undoubtedly create conditions favorable for the development of the larvae of the above-named parasites and many others similar to them. Such conditions would in time result in the occurrence of clinical parasitism in these sheep if measures are not taken to prevent the buildup of infestations with the eggs and larvae of these helminths on the irrigated pastures.

Baby hamster kidney cells infected with foot-and-mouth disease virus contain a new enzyme not found in uninfected cells. This enzyme is capable of catalyzing the synthesis of viral ribonucleic acid in a cell-free system. Serums from infected guinea pigs and cattle contained an antibody which inhibited the synthesis of this viral RNA. The evidence indicates that the enzyme is, in fact, the 3rd antigenic component previously found in infected culture cells and animal tissues.

EXAMPLES OF RECENT ACCOMPLISHMENTS OF THE
STATE AGRICULTURAL EXPERIMENT STATIONS

Disease-free (SPF) cattle. Numerous herds of swine free from specific diseases (SPF herds) have been established as a result of techniques developed at the Nebraska Station. Scientists at Nebraska and other stations now have found procedures successful for establishing cattle free of specific diseases. With specially designed equipment and facilities, unborn calves are removed from the uterus by sterile procedures. Thus, the calves are completely dissociated from certain infectious agents which may be harbored by the mother. Calves are then raised in isolation on sterilized diets to prevent contact with disease agents. The SPF calf is an ideal host in disease and nutrition research since no previous exposure has occurred to dam-transmitted microbial agents. It is anticipated that SPF procedures in cattle will be used primarily in producing animals for research.

A new reproductive disease of cattle discovered. California research workers have recently isolated infectious bovine rhinotracheitis (IBR) virus from fetuses aborted by dairy heifers. The virus causing these abortions was identical to one isolated from aborted calves in Ohio. Abortions have been produced experimentally with this virus by workers in Colorado and California. An IBR virus has also been recovered in fetuses aborted from cows vaccinated against IBR during the gestation period. The discovery of abortion from IBR has important implications, especially in the artificial insemination program, as frozen semen may be a good preservative for infectious agents, particularly viruses. In order to reduce this risk, further research is needed to delineate more clearly this and other possible causes of reproductive failure.

Improved method for detecting cysticercosis in cattle. Human tapeworm carriers in the Southwestern United States sometimes contaminate pastures. This contamination results in cysticercosis in grazing cattle. While this infection is harmless to cattle, the meat from infected animals is a potential health hazard to man. Inspection procedures are effective in keeping such meat out of food channels. Arizona research has found that meat inspection can be even more effective in detecting cysticercosis in cattle by application of a diagnostic test prior to slaughter. In a comparison of the 2 methods, the test identified 26% more infected animals than did the usually prescribed procedures. Inspection procedures have been revised as a result of these findings.

Early vaccination reduces brucellosis control problem. Brucella abortus strain 19 vaccine has provided very effective assistance in controlling brucellosis in cattle. However, the vaccine may interfere with the detection of natural infection if used in cattle above or at the upper level of the recommended minimum age limit of 4 to 8 months. Ohio workers have now established that calves vaccinated at 2 and 3 months of age develop resistance to brucellosis comparable to that of the older animals. Also, the vaccine used at this younger age is less likely to cause reactions which later interfere with the brucellosis test. As a result of these findings, recommendations for brucellosis vaccination are being altered to make use of the improvements offered by this new procedure.

Treatment found for immature liver flukes. Several treatments have been used against liver flukes (Fasciola hepatica) in sheep and cattle, but none has had any significant effect against the immature stages of the parasite present in infected animals. Oregon scientists have found a biphenol compound to be highly effective against both immature and mature stages of the fluke. This is the 1st drug that is effective against immature flukes and should be a significant improvement over other chemical control methods which are used at present.

Starlings found to transmit avian tuberculosis. Investigations of a recent swine tuberculosis outbreak identified it as the avian form usually acquired from poultry. Since there was no exposure to poultry, attention was drawn to the possible role of starlings. Examination of a large number of these birds showed that some were infected with tuberculosis and that they had apparently acquired the disease from a neighboring flock of infected chickens. This is the first recognition of tuberculosis in starlings in the United States and adds another disease agent to several others which this bird is known to disseminate.

Cause of a hemorrhagic enteritis in turkeys determined. Station scientists have identified the causative agents of a disease in turkeys known as "hemorrhagic enteritis." The disease is unique in that a bacterium (a Streptococcus) and a virus act together to produce the disease. An additional unusual finding is that the virus can be maintained in laboratory cultures of the Streptococcus in much the same manner as a bacteriophage. Bacteriophages are viruses which infect bacteria but have not previously been directly involved in causing animal diseases.

Cause of blackhead in turkeys identified. For many years blackhead or enterohepatitis in turkeys has been attributed to the protozoan parasite, Histomonas meleagridis; however, some scientists have questioned the etiologic role of this parasite because of variable results sometimes obtained in experimental transmission. Using germ-free turkeys, Georgia scientists have now established that this protozoan requires the presence of certain intestinal bacteria before disease results. This discovery will provide substantial aid in the search for more effective means to control blackhead.

Influenza discovered as a cause of a respiratory disease in turkeys in the United States. Wisconsin and Massachusetts researchers, working to identify the cause of a new respiratory disease in turkeys, have determined that an influenza virus is the etiologic agent. These are the first identifications of influenza in poultry in the United States. Thus far, field outbreaks have been identified only in turkeys. The disease has been comparatively mild and self-limiting. Experimentally, the virus has not caused visible signs of illness in chickens.

Salmonella typhisuis discovered as one of the causes of enteric diseases in swine in the United States. Bacterial causes of swine enteritis are being studied in Minnesota as a contribution to a regional research project on the causes and control of swine intestinal diseases. Workers have discovered that a rather common type of enteric disease in swine is caused by the bacterium, Salmonella typhisuis. Although evidence indicates that this organism may be widespread, it had not been identified previously as causing disease in the United States. Difficulty in culturing the organism and peculiar biochemical reactions atypical for Salmonella have prevented its recognition in previous cases.

New cultivation method results in discovery of a Mycoplasma causing a mastitis-metritis syndrome in swine. A new cultural medium has been developed for laboratory studies of Mycoplasma, a group of disease-producing organisms some of which are exceedingly difficult to cultivate. The new medium has resulted in isolation of several types of Mycoplasma previously undescribed. One has been recovered from sows affected with a serious disease known as the mastitis-metritis syndrome. The Texas workers have found that this disease can be reproduced experimentally in swine with the new Mycoplasma organism, indicating that it is a cause of this disease.

New unusual route of infection discovered for swine parasite. Infection of baby pigs with the intestinal threadworm, Strongyloides ransomi often occurs within the first few days of life. This early infection results from an infective mechanism unlike that of other livestock parasites. The threadworm finds its way to the mammary gland of the sow and the baby pig acquires infection through ingestion of the worm along with colostrum milk. Work is continuing to trace the migration route of the worm to the sow's mammary gland and to develop methods for interrupting this migration.

Air pollution increases susceptibility to respiratory diseases in poultry. Wisconsin scientists have found that levels of ammonia may build up in poorly ventilated poultry houses during the winter and may damage the respiratory tract of chickens. Resulting changes can make the birds more susceptible to virus infections. With 200 parts per million (p.p.m.) of ammonia in the air, birds showed immediate discomfort and their lungs were damaged in a few days. With 20 p.p.m. in the air, it took more than a month for respiratory damage to show up. However, within 3 days, the birds of either group were more

susceptible to a respiratory infection compared to birds which had not been exposed to ammonia. Twenty parts per million of ammonia in the air is not uncommon in poultry houses. Extended exposure to this level of ammonia could lead to trouble with virus disease infections which are thought to be a part of the airsacculitis problem.



AREA NO. 1 - INFECTIOUS AND NONINFECTIOUS DISEASES OF CATTLE

Problem. Losses from infectious and noninfectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and noninfectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 30.3 scientist man-years. This effort is divided among subheadings as follows:

Vibriosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and through a contract with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreement with Iowa State University, Ames.

Mastitis of Cattle 4.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Epizootic Bovine Abortion 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Fot Rot (Infectious Pododermatitis) of Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological, and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization Against Bovine Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Enteritis of Young Calves 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a contract with the University of Idaho, Moscow.

Bovine Lymphosarcoma 3.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Nebraska, Lincoln, Nebraska and Cornell University, Ithaca, New York.

Respiratory Disease of Cattle (Shipping Fever) 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Brucellosis of Cattle 2.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a P. L. 480 Grant of funds equivalent to \$31,950.00 over a 3-year period.

Paratuberculosis of Cattle (Johne's Disease) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink-Eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Bovine Genital Mycoplasmosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 125.1 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Vibriosis

The National Animal Disease Laboratory (NADL), Ames, Iowa, reports the results of a cultural survey of Vibrio fetus infection in a commercial bull stud. The stud submitted 889 samples of raw semen from 133 bulls for cultural examination. Vibrio isolates were tested for catalase and hydrogen sulfide production, 4.5% NaCl tolerance, 1% glycine tolerance and sensitivity to antibiotics. Vibrio fetus strains isolated from other sources were tested similarly for comparative purposes.

Of the 889 samples of raw semen cultured, 220 (24%) were overgrown with coliform bacteria, 516 were satisfactory for examination, and Vibrio was isolated from 153 (17%) of these. Of the 133 bulls examined, 52 (40%) were infected. The ratio of positive cultures to the total number of satisfactory cultures was 1 to 3. From these results, one would expect to isolate V. fetus from an infected bull in 1 of 4 attempts, considering 25% overgrown cultures.

Biochemical tests on the Vibrio isolates indicated that 47 bulls were infected with V. fetus var. venerealis, 1 bull with V. fetus var. intestinalis, and 3 mount steers with Vibrio species that were nontypable by the methods used. No record was kept of isolations of Vibrio bubulus.

All strains of V. fetus var. venerealis were resistant to penicillin and kanamycin, and all except 6 were resistant to novobiocin. All of these strains were sensitive to streptomycin, chloromycetin, neomycin, and tetracycline. All except 3 were sensitive to erythromycin. These results are consistent with antibiotic sensitivity tests on most strains of V. fetus tested.

(Ames, Iowa) (ADP al-40)

The major object of vibriosis research under Cooperative Agreement No. 12-14-100-880(45), at the New York State Veterinary College has been to improve methods for the diagnosis and treatment of V. fetus carrier bulls. The fluorescent antibody technique, generally in conjunction with improved cultural procedures, has been adapted for this purpose and has proved to be an effective and practical means for the diagnosis of the V. fetus carrier state in the bull. The availability of good diagnostic methods has helped establish workable programs for eradicating vibriosis from artificial insemination cooperatives.

(Ithaca, New York) (ADP al-9 (R))

B. Tuberculosis

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa, as follows:

An intravenous tuberculin test was extensively studied to evaluate more fully its use in detecting bovine tuberculosis. The test was applied to noninfected cattle, cattle artificially sensitized to Mycobacterium bovis, cattle with paratuberculosis, cattle infected with Runyon Group III atypical Mycobacterium, cattle with natural tuberculosis, and cattle experimentally infected with Myco. bovis.

The criteria for a positive reaction in tuberculous cattle were that the peak temperature was reached between 4 to 6 hours following the injection of tuberculin. It remained elevated for 8 hours or longer, exceeding 40 C., and the change in temperature exceeded 1.7 C. In cattle infected with a

Mycobacterium other than Myco. bovis, the temperature change and peak were lower and returned to normal within 8 hours.

Commercially available tuberculin purified protein derivative (PPD) did not cause thermal reactions in tuberculous cattle.

The bacteriocidal activity of benzalkonium chloride was tested against strains of Myco. tuberculosis, Myco. avium, Myco. bovis, Myco. para-tuberculosis, Myco. fortuitum, of Runyon Types I, II, III, and IV and Mycobacteria isolated from lesions in human beings, cattle, pigs, and fowl, of various mycobacterial saprophytes, and strains of Nocardia.

Treatment of the organisms with 0.1% benzelkonium chloride for 1 day was noninhibitory to most strains of Mycobacteria isolated from lesions, but bacteriocidal for saprophytic Mycobacteria and all strains of Nocardia tested.

(Ames, Iowa) (ADP al-13 (R))

Research was continued at Michigan State University, East Lansing, Michigan, under Contract No. 12-14-100-8869(45). Studies were conducted on the McFarland and Heilman method, an in vitro cytotoxic procedure applicable to the study of tuberculo-sensitivity in cattle. Circulating leukocytes from normal rabbits and rabbits sensitized with heat-killed Myco. avium and Myco. bovis (310) were collected, processed, and incubated at 37 C. for 4 days. These cells were stained, examined microscopically, and the percentage of blast forms was determined. This procedure was repeated for 3 successive weeks and the animals were skin-tested by the intradermal inoculation of 0.1 ml. of PPD. The cell cultures were repeated weekly during the following 3 weeks. Results indicated that the variation was too great from animal to animal, and from week to week regardless of sensitization or PPD exposure.

(East Lansing, Michigan) (ADP al-38 (C))

C. Mucosal-Respiratory Disease-Complex of Cattle

Researchers at the National Animal Disease Laboratory, Ames, Iowa, reported as follows:

The role of a bovine viral diarrhea (BVD) virus strain NADL in the etiology and pathogenesis of enteritis of neonatal calves (calf scours) was studied.

Three colostrum-fed calves from dams exposed intravenously to BVD virus at 6, 16, and 25 days prepartum, respectively, had moderate diarrhea persisting till the 8th day of life. The BVD virus was isolated from all 3 calves and persisted up to 93 days in 1 calf, indicating either that BVD was transmitted in utero or via the dam's milk.

Three specific pathogen-free (SPF) calves, permitted dams' colostrum for the first 4 feedings and then given milk replacer, were exposed orally on the day of birth to BVD virus. One calf died of neonatal enteritis 28 hours postexposure and the BVD virus was isolated from several organs. The remaining 2 calves experienced a mild diarrhea persisting to the 8th day of age.

Two calves, which were permitted dams' colostrum ad lib. for 72 hours and then weaned, were exposed orally to BVD virus. Both calves had a mild persistent diarrhea and BVD virus was isolated from the blood for 56 days postexposure.

Of 13 SPF colostrum-deprived calves exposed orally or intranasally on day of birth to the BVD virus, 4 had severe diarrhea and died of neonatal enteritis from 38 hours to 13 days postexposure. Isolations of BVD virus were made from several of their organs at necropsy. All of the 9 surviving calves had a moderate to severe diarrhea frequently persisting for 7 to 10 days, and BVD virus was isolated from the survivors up to 103 days post-exposure.

Several strains of Escherichia coli were isolated from calves after the 2nd day of life, but were neither pathogenic for mice, nor serologically related to strains of E. coli usually associated with outbreaks of calf scours. Four colostrum-deprived SPF calves were exposed orally on day of birth to a strain of E. coli isolated from the intestine of the calf most acutely affected with fatal neonatal enteritis. None of the 4 calves receiving the E. coli had diarrhea. One calf, however, had respiratory distress and died on day 5. Two SPF colostrum-deprived control calves had neither diarrhea nor respiratory distress. Bovine viral diarrhea virus should not be overlooked as a primary cause of the neonatal calf enteritis complex.

Adaptation of the C24V strain of BVD virus (9th passage in primary embryonic bovine kidney (EBK) cells) to the PK-15 cell line was followed by immunofluorescence, and a cytopathogenic effect (CPE) in cell cultures was produced. No CPE was produced in PK-15 cells until the 6th passage, although cell infectivity was shown by immunofluorescence. Subsequent passages caused progressively increasing destruction of PK-15 cells by the virus. From the 6th to the 16th PK-15 passage, no CPE was produced in primary EBK cells when the "adapted" virus was assayed in these cells, although the EBK cells were infected as determined by immunofluorescence.

Attempts were made to produce a chronically-infected cell line by serially transferring C24V-infected PK-15 cells in which the virus had been passaged from 1 to 16 times. Growth of cells was slow in those in which the virus was passaged from 1 to 10 times. Low passaged virus up to the 9th passage completely destroyed their host cells by the 9th transfer. Virus from the 11th to the 19th passages produced chronically-infected cells in which the

supernatant fluid contained "adapted" C24V virus which produced CPE in both EBK and PK-15 cells. From the 20th to the 27th passages, CPE was produced only in PK-15 cells from cell supernatant fluids. Commencing with the lower passaged viruses, they completely destroyed the transferred PK-15 cell line, culture by culture, and all cells had died from viral infection by the end of the 28th transfer. Chronically infected PK-15 cells were not successfully produced with the C24V virus.

An agar staining technique in conjunction with dark ground illumination was used to improve the quality of the precipitin lines in an agar gel double diffusion BVD viral soluble antigen-antibody system. Of 8 colors tested, orange appeared to be the most photogenic. A 1:80 dilution of dye incorporated into melted agar proved to be the most homogenous system when used with glass petri plates. The system achieved good definition of the precipitin lines, desirable resolution, and high quality contrast.

(Ames, Iowa) (ADP al-42)

Iowa State University workers, under Cooperative Agreement No. 12-14-100-5509(45), report the following:

Examination of 640 samples of bovine serum for specific viral antibodies by the plaque reduction technique indicated that 33% contained significant levels of IBR antibody, 66% reacted with BVD virus and 100% neutralized PI 3 virus. It was concluded that there is no way to arbitrarily select 1 screening dilution of bovine serum which would make positive reactions more significant without missing some early responses to clinical infection. The testing of acute and convalescent phase serum samples is imperative to determine the meaning of serological test results.

Primary bovine testicle cell cultures were an excellent host cell system for primary isolation of the common bovine viruses. Examination of 63 specimens from aborted bovine fetuses or other materials permitted the isolation of IBR from 11, BVD from 2 and pseudorabies from 3. Direct isolation attempts were often less time-consuming and performed at less expense to the laboratory than were the serologic titrations.

(Ames, Iowa) (ADP al-42)

D. Mastitis of Cattle

The following results have been reported from the National Animal Disease Laboratory, Ames, Iowa.

Streptococcus agalactiae cultures possess an aerobic pathway for glucose oxidation that is strongly inhibited by cyanide. The products of glucose oxidation by aerobically-grown cells were lactic and acetic acids, acetylmethylcarbinol, and carbon dioxide. Glucose degradation products by

aerobically-grown cells, as percentage of glucose carbon, were 52 to 61% lactic acid, 20 to 23% acetic acid, 5.5 to 6.5% acetylmethylcarbinol and 14 to 16% carbon dioxide. There was no evidence for a pentose cycle or a tricarboxylic acid cycle. Crude cell-free extracts possessed a strong nicotinamide-adenine dinucleotide (NADH) oxidase that is also cyanide-sensitive. Dialysis or ultrafiltration of the crude, cell-free extract results in loss of NADH oxidase activity. Oxidase activity was restored to the inactive extract by addition of the ultrafiltrate or by addition of menadione or potassium ferricyanide. Noncytochrome iron containing pigments are present in cell-free extracts of S. agalactiae.

Strains of Staphylococcus epidermidis isolated from bovine udders were divided into proteinase-positive and proteinase-negative groups based on their proteolytic activity on skim milk agar, Staphylococcus medium No. 110 (Difco) and gelatin. Most of the proteinase-negative cultures produced acetoin, whereas the opposite was true for the proteinase-positive cultures. A further subdivision of the cultures in each group could be made by using Baird-Parker's biochemical subgrouping scheme. The proteinase-positive cultures were also subdivided by serologic typing of their proteolytic enzymes into 5 groups: B, F, G, H, and NR, a nonreacting group. The colonies formed by the cultures were classified into 5 types, each consisting of 2 to 4 forms. Some staphylococcal cultures that were coagulase-positive were more closely related to S. epidermidis than to S. aureus, based on their biochemical reactions and the serologic grouping of their proteolytic enzymes.

(Ames, Iowa) (ADP al-15)

Scientists at the University of California, Davis, under Cooperative Agreement No. 12-14-100-2380(45) report the following:

Streptococci were weakly antigenic in rabbits and produced both nonspecific and cross-reacting precipitins. Antigens from Streptococcus dysgalactiae seemed to be the most widely distributed among the Streptococci. Precipitins to S. dysgalactiae were the most widespread in cattle serums tested, regardless of previous history of mastitis. Precipitins to S. agalactiae, on the contrary, were found in only 1 cow that had a 2-year history of udder infection with S. agalactiae.

In the study of bactericidal activity of bovine body fluids, colostrum from young first-calf heifers was only weakly bactericidal for our serum-susceptible strain of Aerobacter aerogenes whether complement was present in the test system or not. Colostrum from an older animal was bactericidal when the test system was supplemented with specifically absorbed complement.

The phagocytic activity of leukocytes which enter the mammary gland during inflammation, can play a significant role in protecting the gland against permanent infection with several udder pathogens. In comparison of mammary response to A. aerogenes inoculations, a cow during the leukopenic

state had a mild inflammatory response and a normal cow had a severe response. Cellular emigration into the milk and swelling of the inoculated quarter were minimal under the leukopenic condition. Infection was eliminated from the quarters in each case.

(Davis, California) (ADP al-15)

E. Epizootic Bovine Abortion

Research at the University of California, Davis, under Cooperative Agreement No. 12-14-100-9084(45) produced the following results:

Vaccination appears to be of questionable value in controlling epizootic bovine abortion. In fact, there is some doubt whether cattle become immune following natural infection with this agent.

The possibility that viruses common to cattle, with the exception of the viruses of infectious bovine rhinotracheitis and bovine viral diarrhea, might cause abortion simulating that produced by the epizootic bovine abortion agent has been largely excluded.

(Davis, California) (ADP al-21 (R))

F. Bovine Pulmonary Adenomatosis

The National Animal Disease Laboratory reported that projects were completed describing the pathologic changes in cattle resulting from inhalation and rumen insufflation with nitrogen dioxide. Inhalation of the gas caused methemoglobinemia, severe dyspnea, and death. Pulmonary lesions consisted of hyperemia, edema, hemorrhage, fibrin deposition, hyperplasia of the respiratory epithelium, obliterative bronchiolitis, infarction, and emphysema. Intraruminal administration of nitrogen dioxide resulted in severe necrosis of the rumen but only minor changes in the lungs. It was concluded from these studies that nitrogen dioxide is probably not involved in bovine pulmonary adenomatosis.

(Ames, Iowa) (ADP al-24)

G. Leptospirosis

Scientists at the National Animal Disease Laboratory, Ames, Iowa report that the currently available bacterins neither block the dissemination of leptospires among animals nor prevent the contamination of our recreational waters. Furthermore, their use is costly (about \$20 million annually) and repetitive use frequently causes losses from anaphylaxis. Consequently, some authorities have questioned the justification for their continued use.

Attempts to identify the protective factor and to derive an effective and practical immunizing agent have continued. Although a soluble protein of

less than 1 million molecular weight immunizes hamsters against lethal leptospirosis, immunity to the more important renal form apparently requires a living vaccine. After exposure to gamma rays or streptomycin, nonreplicating leptospires induced considerable immunity in hamsters and swine to renal leptospirosis, but the best protection was by a living, avirulent culture. The new vaccine immunized cattle without causing any evidence of infection. Further studies on safety, potency, and duration of immunity are in progress.

(Ames, Iowa) (ADP al-25)

After a method was devised for consistently initiating renal leptospirosis in cattle, streptomycin was tested for efficacy. Three daily doses of 25 mg./kg. of body weight were effective; trials to determine if smaller doses are effective are in progress.

Because of the cost and labor involved in parenteral administration of streptomycin to cure renal leptospirosis, the search has continued for drugs which would be effective when added to the feed or drinking water. A new antibiotic agent, rifamycin, was tested and was ineffective in vivo.

(Ames, Iowa) (ADP al-26)

In contrast to earlier results with laboratory-adapted strains, glucose did not stimulate the growth of a virulent strain of Leptospira pomona. Comparisons of virulent and avirulent L. pomona did not confirm suggestions by other investigators that hemolysin or lipase are leptospiral virulence factors. In addition, leptospiral lipids and a newly found toxic factor(s) were demonstrated in both virulent and avirulent leptospires. However, enzymatic activity on lecithin (lecithinase) was absent in avirulent L. pomona.

(Ames, Iowa) (ADP al-41)

H. Enteritis in Young Calves

The Caldwell Veterinary Research Laboratory, University of Idaho, under Contract No. 12-14-100-8300(45), reported completion of the first phase of studies on resistance to enteritis among calves. Forty beef cows and 2 beef bulls have been purchased, brought to the station, bred, and extensively studied both culturally and serologically. Research on the 2nd phase of this study has begun. Levels of serum proteins and specific antibodies (E. coli serotype 026 and serotype 08) in experimental cows and their calves have been determined. Levels of prenursing colostrum globulins have also been determined. These data are being correlated with susceptibility of calves to oral inoculation with E. coli or other enteritis-producing agents.

(Caldwell, Idaho) (ADP al-29 (C))

I. Bovine Lymphosarcoma

The New York State Veterinary College, Cornell University, Ithaca, submitted the following report under Cooperative Agreement No. 12-14-100-5583(45).

Extensive study of bovine lymphosarcoma tissues from 11 spontaneous cases has been made by electron microscopy of nonconcentrated and concentrated material. No virus particles have been observed in these materials.

Six newborn calves, 4 of which received total body irradiation failed to develop leukemia under the defined experimental conditions.

(Ithaca, New York) (ADP al-30)

The Agricultural Experiment Station, University of Nebraska, Lincoln, reported the following under Cooperative Agreement No. 12-14-100-5590(45).

Cell cultures have been prepared from thymic tissue and lymph nodes from a steer with the splenic form of leukemia. Attempts are being made to develop stable cell lines of these cultures. An electron microscopic study of these cells thus far has failed to reveal definite virus-like particles.

These cells support the growth of the viruses of infectious bovine rhinotracheitis and pseudorabies.

Results to date indicate that specific antigen-antibody has not been demonstrated in bovine lymphosarcoma.

(Lincoln, Nebraska) (ADP al-30)

The Animal Health Division, ARS, is cooperating with the Animal Disease and Parasite Research Division in a study of the epidemiology of bovine lymphosarcoma. The activities are centered at the University of Minnesota and the University of Pennsylvania. Data are gathered on herds known to have this disease as well as those free of it. Hematological studies are being conducted and virus isolations are being attempted. Husbandry and environmental factors are also being investigated in some herds.

(Ames, Iowa) (ADP al-30)

J. Respiratory Diseases of Cattle

The following report was submitted from workers at the National Animal Disease Laboratory, Ames, Iowa.

High molecular weight dextran sulfates markedly enhanced infection of mice inoculated intraperitoneally with Pasteurella haemolytica and were almost as effective as gastric mucin. Related compounds, such as heparin or chondroitin sulfate, were less effective. Lung washings, obtained by

perfusion of the respiratory tract with 0.85% NaCl, were also effective in enhancing infection, but bovine nasal mucus samples were not. Similar effects were obtained with guinea pigs, hamsters, and suckling mice when inoculated with dextran sulfate (molecular weight 5×10^5) and Past. haemolytica.

(Ames, Iowa) (ADP al-31)

Calves exposed to aerosols of parainfluenza-3 (PI-3) virus had a febrile response and signs of respiratory infection. Those exposed to Past. haemolytica in the same manner had a very brief febrile response and mild signs of respiratory infection. Calves exposed to both PI-3 virus and Past. haemolytica via aerosol had a prolonged febrile response, marked signs of illness, and lung lesions.

A modified hemagglutination inhibition test was developed, including a method to remove nonspecific inhibitors of hemagglutination from the serum before testing. With this technique, "negative titer" levels were established in fetal calf serums and serums from nonexposed colostrum-deprived, isolation-raised calves.

(Ames, Iowa) (ADP al-39)

K. Brucellosis of Cattle

At the National Animal Disease Laboratory, Ames, Iowa, a study was conducted to determine the resistance of pregnant heifers to Brucella abortus strain 2308 following vaccination with strain 19 at 2 or 3 months of age. Of the 10 heifers vaccinated at 2 months of age, 3 (30%) became infected, and 2 of these aborted. Three of 8 (37.5%) heifers vaccinated at 3 months of age became infected, and 2 aborted. Of the 6 pregnant nonvaccinated controls, 4 (66.7%) became infected and aborted. In addition, 1 nonpregnant, non-vaccinated heifer was infected at the time of necropsy.

Post vaccinal agglutinin titers were lower and receded to a negative status sooner in those cattle vaccinated when 2 or 3 months old than in those vaccinated at 4 months or older. The resistance to infection of 2- and 3-month old vaccinated cattle compared favorably with resistance previously found in cattle vaccinated when 4 to 8 months old.

(Ames, Iowa) (ADP al-32)

The Ohio Agricultural Experiment Station, Wooster, under Cooperative Agreement No. 12-14-100-5490(45), found that postvaccinal agglutinin titers were lower and receded to a negative status (1:50 or lower) sooner in those cattle vaccinated when 2 and 3 months old than in those vaccinated when 4 to 8 months old. Under the conditions of these experiments, with a small group of animals, the resistance to infection of 2- and 3-month-old vaccinated cattle compared favorably with resistance previously found in cattle

vaccinated when 4 to 8 months old.

(Wooster, Ohio) (ADP al-32)

Workers at the University of Wisconsin, Madison, under Cooperative Agreement No. 12-14-100-928(45), reported that the early antibody produced in primary response to strain 19 vaccine was 2-mercaptoethanol-sensitive and had complement fixing activity. This 2-mercaptoethanol-sensitive antibody was rapidly augmented by antibody which was stable to treatment with 2-mercaptoethanol. The early antibody was also inactivated by treatment with rivanol or by heating at 65 C. for 15 minutes. Later serum samples contained agglutinins, which were not affected by such treatment.

In animals which were revaccinated with strain 19, the earliest samples contained 2-mercaptoethanol, rivanol, and heat-stable and labile agglutinins.

With animals vaccinated at the recommended ages of 4 to 8 months, as the titers of antibody fell, the supplementary tests studied did not identify fewer animals as reactors any sooner than the standard tube agglutination test.

In the course of time following vaccination of cattle over the recommended ages, the complement fixation test consistently identified fewer animals as reactors sooner than the other supplemental tests studied. By 1 year after vaccination of this group, there were no significant differences in the number of reactors identified by any of the supplemental tests.

A small group of ewes and 1 ram naturally infected with B. abortus were acquired during the year and maintained in isolation for the entire year. Pregnant ewes lambed or aborted and have been bred to a normal uninfected ram. Serological and bacteriological investigations have been made over the course of time. The ram which was infected and shedding B. abortus in semen when acquired was allowed to mate with 10 normal uninfected ewes. All of the observations have not been completed but several will be summarized here. 1) There was no transmission of B. abortus from naturally-infected ewes to a normal ram allowed to mate with them. 2) Transmission did not occur from the naturally-infected ram to the normal ewes with which he mated. The ram had a lowered antibody titer and was not shedding Brucellae in semen before mating. 3) Lambs infected in utero synthesized antibody in utero. This fact was established by preventing them from obtaining colostrum. The immunoglobulin type is being investigated. The antibody did not fix complement but was sensitive to mercaptoethanol. 4) Typical B. abortus type 1 was isolated.

(Madison, Wisconsin) (ADP al-32)

Under a P. L. 480 Grant, investigations on the immunizing effect of brucella cell wall continued at the Hebrew University, Hadassah Medical School, Jerusalem, Israel. The immunizing potency of cells, cell walls, and cell

wall preparations of B. abortus, B. melitensis, and B. suis, against infection with B. melitensis, was determined in mice. Vaccines were administered intact or after disintegration by ultrasonic vibration, at 3 dosage levels: 40 µg., 4 µg., and 0.4 µg. Cell walls proved more effective than intact cells in all cases. The most potent preparation was a vaccine consisting of 40 µg. B. abortus cell walls, disintegrated ultrasonically. When the vaccines were tested simultaneously, B. melitensis cell wall vaccine was the most effective.

(Jerusalem, Israel) (A10-ADP-6)

At the University of Minnesota, St. Paul, under Cooperative Agreement No. 12-14-100-866(45), researchers reported the following:

Further studies of the reported epizootic of B. abortus infection in a flock of sheep indicated that infection may be maintained in ewes for more than one year. Infection was transmitted to many of the spring lambs although the rate of abortion was greatly reduced.

Results of an experiment to evaluate a commercial vaccine for bovine brucellosis indicated that it is not as "non-agglutinogenic" as previously reported by European investigators. However, it does produce less detectable agglutinin response than does strain 19 vaccine.

Studies of antibody response in swine to natural and experimental infections with B. suis have been continued to evaluate the diagnostic procedures for swine brucellosis. Results thus far indicate a need for more research to refine diagnostic methods and to interpret serologic procedures.

(St. Paul, Minnesota) (ADP al-32)

L. Paratuberculosis of Cattle (Johne's Disease)

The National Animal Disease Laboratory, Ames, Iowa, reported that tissue specimens from 148 cattle in a herd of 1000 in which paratuberculosis was a problem were made available for laboratory examination. Portions of the uterus, ileocecal valve, ileum, and adjacent lymph nodes were examined and cultured for the presence of Mycobacterium paratuberculosis. Fourteen out of 18 specimens of uterine infection were in cattle that were infected but had no outward signs of disease.

Blood chemistry studies were made in a herd of cattle infected with Johne's disease. All cattle were examined physically for clinical signs of the disease and bacteriologically at necropsy for evidence of infection.

Serum levels of inorganic phosphorous, calcium, sodium, potassium, magnesium, and alkaline phosphatase activity were determined. The values obtained from noninfected cattle and infected cattle not showing signs of disease were similar to published values for normal cattle, but infected

cattle with clinical signs of disease had higher levels of phosphorous and phosphatase activity than normal cattle.

(Ames, Iowa) (ADP al-35)

M. Bovine Genital Mycoplasmosis

At the National Animal Disease Laboratory, Ames, Iowa, Mycoplasmata were relatively common in processed and nonprocessed bull semen. They were also isolated from vaginal mucus, an aborted fetus, and preputial mucus.

Thirteen of the 14 semen isolates resembled Mycoplasma bovigenitalium. Three isolates, 1 each from semen, vaginal mucus, and prepuce, resembled Myco. laidlawii. Two isolates, 1 each from vaginal mucus and an aborted fetus, may represent a new species. The cytopathic effect on primary cell cultures was valuable in the differentiation of these Mycoplasmata. Growth-inhibition test results suggest that 8 serotypes may be represented among these isolates.

Intrauterine exposure of heifers to Mycoplasmata of semen origin produced little or no clinical effect. At necropsy, samples from the genital tract were culturally negative for Mycoplasmata. Mycoplasmata could not be isolated from vaginal mucus beyond 1 day postexposure. Semen isolates were able to establish in the preputial cavity of exposed bulls. Vulvovaginitis was produced when heifers were bred by natural service to infected bulls; however, Mycoplasmata were not isolated from the vaginal mucus of these heifers. In contrast, intrauterine exposure to M. agalactiae var. bovis produced signs of infertility and vaginal infection was proved.

Significant gross and microscopic inflammatory changes of the mesovarium were found in 3 of 5 heifers exposed to 1 group of semen isolates and in both heifers exposed to M. agalactiae var. bovis. Chronic salpingitis was also observed in 1 heifer exposed to M. agalactiae var. bovis.

(Ames, Iowa) (ADP al-33)

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AREA NO. 2 - INFECTIOUS AND NONINFECTIOUS DISEASES OF SWINE

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than \$200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventive procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 12.7 scientist man-years. This effort is divided among subheadings as follows:

Hog Cholera 5.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a contract with the University of Nebraska, Lincoln.

Erysipelas 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Brucellosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Abscesses 1.0 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Colorado State University, Fort Collins, Colorado, and Purdue University, Lafayette, Indiana.

Atrophic Rhinitis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University, Lafayette, Indiana, and the University of California, Davis.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 35.6 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Hog Cholera

Evaluation of commercial hog cholera virus vaccines

Thirty-three commercial modified live hog cholera virus vaccines of all 3 types (lapine, porcine, and tissue culture origin) were studied to determine whether the vaccine spreads to nonvaccinated pigs and whether the vaccines transmit immunizing virus by contact.

Of the 33 vaccines studied, 5 were lethal to 5 of 10 vaccinates. Nine of the vaccines transmitted lethal hog cholera virus by contact, and 8 of them did so even with the simultaneous use of antiserum. Twenty-five of the vaccines transmitted immunizing virus by contact and 22 of them with the simultaneous administration of antiserum. Eight of the vaccines transmitted both lethal and immunizing virus and 7 of them also with the use of antiserum simultaneously.

(Ames, Iowa) (ADP a2-17(C))

Adsorption and elution of HCV on powdered iron oxide

The additional area of investigation was concerned with a practical method for partial purification of cell culture-attenuated hog cholera virus (HCV) with good recovery of original virus infectivity. Using methods and the ferric oxide (Fe_2O_3) described by Warren *et al.* (Proc. Soc. Exptl. Biol. Med. 1966. 121:1250-1253) HCV was adsorbed to Fe_2O_3 but was not eluted by saturated sodium phosphate or carbonate solutions. However, after adsorption to Fe_2O_3 and subsequent washing in 0.85% saline, 50-100% of the original infectivity was eluted by 0.01-0.001 M NaCN using 2-4 batch-wise elution steps in centrifuge bottles. The ability of NaCN to dissociate Fe_2O_3 -HCV complexes is pH dependent, being most effective between pH 9.6-10.0. Although HCV is rather labile at this pH, brief exposure in this procedure did not result in serious loss of infectivity. Cyanide ions were removed by dialysis against buffered saline, and infectivity endpoints were determined by fluorescent-antibody plaque assay in cell cultures. Micro-Kjeldahl N determinations show substantial decreases in nitrogenous matter with increases in a PFU/N ratio. The procedure has potential for further purification and concentration via a repetitious series of adsorptions and elutions.

(Ames, Iowa) (ADP a2-17(C))

The purification and concentration of hog cholera virus

Development of a batch-type chromatographic procedure with magnetic ferric oxide (MFO) to partially purify cell cultured HCV was described. The findings indicate that infectious HCV adsorbed by MFO from isotonic solutions was subsequently eluted under conditions of low ionic strength and relatively high pH (ca. 9.7). Dilute solutions of sodium cyanide (0.01 M) and ammonium hydroxide (0.003 M) effectively dissociated MFO-HCV complexes. Fifty to 75% of the original HCV infectivity could be recovered with such a procedure concomitant with a 90-95% reduction of extraneous organic nitrogen.

MFO-purified HCV was concentrated by rate-zonal type density gradient centrifugations in buffered solutions of cesium chloride and sucrose. Hog cholera virus was most stable in the buffered sucrose solution. Concentrated MFO-purified HCV was then subjected to isopycnic gradient experiments in cesium chloride and sucrose. As a result of procedures used to prepare HCV for isopycnic centrifugation in sucrose, all infectivity was lost. However, 2 successful isopycnic centrifugations of HCV in cesium chloride indicated a buoyant density of 1.14-1.15 gm./ml. for the virus.

The isopycnic density fraction containing the highest HCV infectivity titer was negatively stained with phosphotungstic acid and examined with an electron microscope. Electron micrographs were obtained which show characteristic virus-like particles 40-50 mμ in diameter and large numbers of unidentified 12-15 mμ entities. The 40-50 mμ particles were surrounded by a poorly defined asymmetric sac-like membrane. Both particles were aggregated by interaction with specific HCV antibody. In the antibody-aggregated masses, the 40-50 mμ particle had spherical to hexagonal symmetry but visual evidence of the membrane was obscured. Although it is most likely that the 12-15 mμ entity is a degradation product of the 40-50 mμ particle, its true identity remains speculative. However, it is most probable that the 40-50 mμ particle enveloped by the sac-like membrane is an image of the intact, infective virion of HCV.

(Ames, Iowa) (ADP a2-17(C))

Cytogenetic characteristics and susceptibility of mammalian cells to infection with virulent and modified hog cholera viruses

In connection with a host range study of potential hosts of HCV, chromosome preparations were made from the cultured kidney cells of a young female peccary. Although the peccary is a "pig-like" animal, its diploid chromosome number was determined to be 30, whereas the number for the domestic pig is 38.

Dissimilarities have been observed in 2 PK-15 swine kidney cell lines. As these 2 cell lines both have modal chromosome numbers of 37, means to distinguish them were sought. It was determined that the PK-15(ATCC) cell line has 4 marker chromosomes, whereas the PK-15(NADL) line has only 2 marker chromosomes. Furthermore, whereas the PK-15(NADL) line will propagate optimally in a medium with reduced serum supplement, the PK-15(ATCC) line propagates poorly in the same medium with a low serum content. If confusion arises regarding these two PK-15 cell lines, the differences in their nutritional requirements and in their marker chromosomes should assist in identifying them.

The PK-15(ATCC) cell line was infected with the Ames strain of virulent HCV and then subcultured through a series of 41 passages. Noninfected cells of identical passage levels were studied in parallel. Numerous types of cell damage were observed in the infected cells.

Twenty-four primary cell cultures, 14 low passage cell line cultures, and 13 high passage established cell line cultures, derived from 7 orders and 29 species of mammals, were exposed to virulent and modified HCV. The presence of infection was determined with fluorescent antibody. Twelve of 24 primary cell cultures were susceptible to both virulent and modified HCV. Ten of the 14 low passage cell line cultures were susceptible to virulent HCV, and 13 of the 14 cultures were susceptible to modified HCV. Seven of the 13 high passage, established cell line cultures were susceptible to virulent HCV, and 8 of the 13 cultures were susceptible to modified HCV.

(Ames, Iowa) (ADP a2-17(C))

Extraction of infectious nucleic acid from hog cholera virus and electron microscopic observations of cells infected with hog cholera virus

Several preparations have been made from HCV-containing supernatant culture fluids, HCV-infected cultured swine kidney cells, and from concentrated HCV-containing supernatant fluids. Several of the extracts have yielded typical nucleic acid peaks in the spectrophotometer, but as yet we have not been successful in infecting HCV-susceptible cells with these extracts. Our present efforts are directed toward obtaining HCV with higher infectivity titers.

Cultured swine kidney cells, acutely and persistently infected with HCV, were prepared for electron microscopy and examined. Thus far no evidence of virus-like particles has been observed.

(Ames, Iowa) (ADP a2-17(C))

Production, concentration, and purification of hog cholera virus

A plaque assay system was investigated for the purpose of enumerating infective units of the noncytopathogenic Ames strain of HCV. In the absence of apparent cytologic changes, discrete foci of infection were made evident by fluorescent antibody staining. The localization of primary plaques was accomplished by incorporating anti-hog cholera serum into the nutrient medium of infected cell cultures. Plaque size was directly related to incubation time and inversely related to the concentration of antiserum. It was concluded that the primary mechanism for the spread of infection in cell culture is through the dissemination of extracellular HCV. However, a concentration of antiserum in excess of that required to prevent the formation of secondary plaques still allowed contiguous peripheral extension of primary plaques. Thus, the possibility of direct cell-to-cell transfer of infectious virus was suggested.

The reliability of the fluorescent plaque assay was confirmed by the observation indicating that in the presence of a sufficient concentration of antiserum, the number of plaques did not increase with continued incubation of infected cultures. In addition, a linear relationship was found between HCV concentration and plaque counts, and, finally, titers obtained by the plaque assay were essentially the same as 50% cell culture infective dose titers.

(Ames, Iowa) (ADP a2-17(C))

B. Swine Erysipelas

Information as to how the causative organism of swine erysipelas is shed by clinically affected pigs would be useful in the formulation of effective measures for the prevention and control of herd outbreaks. However, a complete determination of the routes and times of elimination of Erysipelothrix from infected pigs has not been reported. Therefore, a study was made of 18 experimentally-infected pigs to determine the routes by which the organism is eliminated into the environment, and the times that elimination occurs during the course of clinical illness.

Erysipelothrix was shed frequently by 17 pigs that had mild to severe signs of generalized infection after exposure to the organism, and shedding was preceded or accompanied by detecting the organism in the blood. The urine and feces were the earliest and most persistent routes of elimination, and often contained the organism before visible evidence of erysipelas was present. The organism was recovered less often from the mouth, nasal passages, skin surfaces, and conjunctival sacs, and generally appeared after erysipelas was visibly evident. One exposed pig did not shed the erysipelas organism nor was the organism found in the pig's blood.

(Ames, Iowa) (ADP a2-21)(ADP a2-15)

C. Swine Brucellosis

The early pathogenesis of brucellosis was investigated in 28 swine allotted to 4 groups. The swine, mainly boars, were exposed to viable Brucella suis via the conjunctival route. Swine in 2 groups were exposed to a strain of B. suis, type 1, and those in the other 2 groups were exposed to different strains of B. suis, type 3. Clinical, serologic, pathologic, and extensive bacteriologic examinations were conducted.

In 4 boars, severe clinical signs could be correlated with the occurrence of gross pathologic changes in accessory genital organs. Brucella agglutinins appeared in diagnostic amounts in the serum of infected swine 1 week post-exposure, reached their maximal level about 2 weeks postexposure, and then gradually receded. Agglutinins were also in exudate from seminal vesicles that had gross lesions. Gross lesions attributable to B. suis infection were limited mainly to seminal vesicles and the regional lymph nodes. Differential blood leukocyte counts were not affected by brucellosis. Infection was most widespread in the body during and soon after periods of sustained brucellemia. Maximal dissemination of infection extended approximately 2 through 4 weeks postexposure. Upon bacteriologic examination at necropsy, B. suis was isolated chiefly from the lymph nodes and the genital system.

There was no major qualitative difference in the characteristics of the disease produced by the 2 different biotypes of B. suis. However, quantitative differences attributed to differences in B. suis strains were observed.

(Ames, Iowa) (ADP a2-16)

D. Swine Abscesses

On 5 consecutive days, each of 5 groups of specific pathogen-free (SPF) pigs (hysterectomy derived) were fed a strain of Lancefield's serologic group E Streptococci. One pig from each of the 5 groups and from a control group was skin-tested on day 14 after the last feeding of the organisms, and one of the remaining pigs in each group was tested on day 28, 42, 56, and 70. Blood samples for serum were obtained also on these days. On day 6 after being skin tested, each pig was euthanatized and physically examined for abscesses.

Antigens for the skin and precipitin tests were prepared by concentration of the culture filtrates (CCF) of each of 7 strains of group E Streptococci, 5 of which had been used to expose the pigs.

Twenty of the 25 principals had abscesses, and group E Streptococci were removed from representative abscesses of 19.

The results of the skin test indicated that exposure by feeding group E Streptococci induced sensitivity to the antigens whether or not the principals had visible abscesses.

No single antigen reacted with all the serums that were recorded as positive in the precipitin test. The serums of all pigs affected with abscesses, however, reacted with 1 or more of the antigens, although the serum of 1 visibly unaffected pig reacted only with the homologous antigen.

The nature of the reactions in the precipitin test (relative to CCF antigens) indicated that there were quantitative as well as qualitative differences among the strains of group E Streptococci.

Blood samples, mandibular lymph nodes, and visible abscesses were collected from 73 market pigs at a packing house. The pigs were procured in 4 groups; groups I and III were designated before slaughter as nonaffected with abscesses and groups II and IV as affected. The collected tissues were examined bacteriologically, and the serums tested for precipitins using 7 CCF antigens prepared from different strains of group E Streptococci.

No abscesses were found and no group E Streptococci were isolated in the pigs of groups I and III. Abscesses were found in 16 of 20 pigs of groups II and groups E Streptococci were isolated from representatives of 15 pigs; group C Streptococci were isolated from the remaining representative abscess. Of 21 pigs of group IV, 1 pig had an abscess from which group E Streptococci were isolated.

The serums of 9 of 56 pigs in which no abscesses were found reacted with 3 or more of the 7 CCF antigens. All serums from 16 pigs that had abscesses associated with group E Streptococci, and from the 1 pig that had an abscess from which group C Streptococci were isolated, reacted with 3 or more of the antigens. A CCF antigen prepared from the isolated group C Streptococci did not react with the serum from the pig from which it was isolated.

(Ames, Iowa) (ADP a2-19)

In the cooperative research at the Agricultural Experiment Station, Fort Collins, Colorado, 4 swine were fed a culture of the group E Streptococcus to verify its abscess-inducing capability. Each of these 4 swine developed neck (jowl) abscesses. One of these infected swine was subsequently penned with 10 healthy weaned pigs to study the dynamics of transmission of the group E Streptococcus. Prior to this event normal values for body temperature and leukocyte counts were obtained from the 10 pigs.

(Fort Collins, Colorado) (ADP a2-19(CA))

E. Atrophic Rhinitis

Attempts to isolate and identify the causative agent(s) of transmissible atrophic rhinitis (AR) during the reporting period were unsuccessful. No evidence of respiratory disease has been observed and no isolations of viruses in tissue cultures have been made from exposed pigs, but the transmissibility of the condition repeatedly has been demonstrated by aerosol instillation to SPF pigs. Continued studies were carried out to measure viability changes in infectious materials stored for different lengths of time. Such materials produced turbinate atrophy even after 21 months of storage in liquid nitrogen, but a decline of infectivity was observed in continued storage. A total of 112 hysterectomy-derived, colostrum-deprived pigs obtained from SPF-certified Iowa swine herds were used during the reporting period. Bordetella bronchiseptica and Mycoplasma spp. were isolated most frequently from infectious materials and from the exposed pigs at the termination of the experiment. Seven to 15-day-old SPF pigs were exposed intranasally to chicken embryo-passaged cultures of these organisms, both alone and in combination. The organisms were infective for swine and recovered in heavy growth from the nasal and tracheal exudate at necropsy, but did not cause turbinate atrophy.

Calcium deficiency in the pathology of AR has been investigated. The attempt to repeat the experimental result of the workers at Cornell University failed. Feeding a low calcium content diet did not aggravate the clinical signs of AR, and feeding a high calcium diet did not prevent development of turbinate atrophy in exposed pigs. Noninstilled pigs sustained on low calcium diet for 6 1/2 months did not develop gross lesions of AR, yet the severe signs of calcium deficiency were apparent. The result indicated that inflammatory processes are the most important factors in the development of turbinate atrophy and the condition is not caused by calcium deficiency.

(Ames, Iowa) (ADP a2-20)

F. Transmissible Gastroenteritis

In cooperative studies at Purdue University, work was done on the sequential development of lesions caused by transmissible gastroenteritis (TGE) virus. The lesions were studied both in pigs killed at frequent intervals after inoculation and in surgically prepared, isolated segments of jejunum. The techniques used were standard histology, fluorescence microscopy, electron microscopy, and virus titrations. Work was also started on the use of fluorescence microscopy as a diagnostic technique. None of this work was published nor could it be considered completed.

(Lafayette, Indiana)(ADP a2-23)

Studies are in progress at the University of California on TGE. This disease reappeared on the premises from which a noncytopathic TGE agent was recovered in 1965. The diagnosis was confirmed by inoculation of susceptible pigs.

Exposure of pregnant sows and gilts to suspensions prepared from the intestine of infected baby pigs was successful as a control measure.

An enterovirus of the Teschen-Talfan group isolated from California swine was used to expose sows in late pregnancy to determine whether their newborn pigs would develop persistent infections. Sows exposed during the last 2 weeks of pregnancy did not pass the infection to their pigs, so studies of exposure at an earlier stage of pregnancy are contemplated.

During the surveillance of larger swine herds (1000-6000 head) for enterovirus-induced encephalitis, another type of encephalitis also caused severe losses. It was then established that pseudorabies was widespread in California, garbage-fed swine and was complicating diagnosis.

(Davis, California) (ADP a2-23)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Hog Cholera

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AREA NO. 3 - INFECTIOUS AND NONINFECTIOUS DISEASES OF SHEEP AND GOATS

Problem. Infectious diseases of sheep and goats in the United States cause an estimated annual loss of 15 million dollars. Noninfectious diseases are estimated to cause an additional 3 million dollar loss annually. The cause of some of these diseases is known; others have more than one causative agent contributing to produce the effects seen in field cases. Environmental, genetic, and unknown factors appear to play a part in some diseases. The natural reservoirs of the known infectious agents have not been fully determined. Fundamental information on methods of transmission and means of prevention are needed for many of these diseases. Vaccines and other immunizing products are available for some diseases of sheep but not for others. Some of these products might be improved. Prevention, control, or eradication of disease is necessary for economic and efficient sheep and goat raising. Because of lack of accurate, rapid diagnostic techniques, infectious diseases often get a substantial start in a band or flock before they are recognized, partly because they are easily confused with non-infectious diseases.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving veterinarians, biochemists, microbiologists, and pathologists engaged in both basic studies and the application of known principles to the solution of infectious and noninfectious diseases of sheep and goats. Research is being conducted on the diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 6.5 scientist man-years. This effort is applied as follows:

Bluetongue 4.0 at the Ectoparasite Vector Research Laboratory, Denver, Colorado.

Vibriosis 0.2 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado, Montana, and Utah Agricultural Experiment Stations.

Paratuberculosis 0.2 at the National Animal Disease Laboratory, Ames, Iowa.

Ulcerative Dermatitis 0.1 under a cooperative agreement with the Colorado Agricultural Experiment Station, Fort Collins.

Toxicological Effects of Oxalate-Containing Plants 1.0 at the Poisonous Plant Research Laboratory, Logan, Utah.

Clinicopathologic and Preventative Aspects of Poisonous Plants 1.0 at the Poisonous Plant Research Laboratory, Logan, Utah.

Scrapie at the Agricultural Research Council Field Station, Compton, Berkshire, England, and the Moredun Institute, Edinburgh, Scotland, through 2 grants of P.L. 480 funds.

Pulmonary adenomatosis at the College of Veterinary Science, Mathura, U.P., India initiated this year through a grant of P.L. 480 funds.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 21.9 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Bluetongue

At the Division's Ectoparasite Vector Research Laboratory, Denver, Colorado, the following research was accomplished:

Cytodifferentiation of the salivary glands of *Culicoides variipennis*, a vector of bluetongue disease

The salivary glands undergo a syncytium, that is, they contain no inter-cellular boundaries. The nuclei are scattered about the cytoplasm at random. There are large, dark-staining masses occupying the cytoplasm, presumably high energy storage products. After the insect takes a blood meal, the cytoplasm undergoes differentiation somewhat similar to that seen in a mammalian embryo early in gestation. The basement membrane invaginates and gives rise to the mitochondria, endoplasmic vesicles, and Golgi apparatus. The dark-staining masses gradually disappear while the duplicating membranes penetrate the mass until the entire centers are filled with microvilli. At this stage, the space originally occupied by the large, dark-staining masses coalesces, the gland obtains a lumen, and cellular differentiation is complete. It is necessary to study the normal cytology of the gland prior to describing the replication of bluetongue virus in the gland cells.

Observations of bluetongue virus in cultured cells

Some of the developmental cycle of bluetongue virus (BTV) was observed in thin sections of cultured cells. Mature BTV was first observed in cultured cells 15 hours after the cultures were inoculated. The virus develops in association with vesiculated bodies in the cytoplasm. These vesiculated bodies developed from the nuclear envelope, plasma membrane, endoplasmic vesicles, mitochondria, and Golgi vesicles. The mature virus was often observed in association with inclusion bodies that were positive for

ribonucleic acid. Mature BTV was never observed in association with the nucleus. Bluetongue virus is apparently coded to stimulate all membranous elements of the cell to undergo extensive reduplication. These membranes have an altered protein structure since they fluoresce when stained by BTV-specific antibody-fluorescein conjugates. Bluetongue virus was observed to be associated with the Golgi apparatus, mitochondria, and endoplasmic reticulum. This study is the important first step in determining the fate of BTV in the host animal and understanding the reservoir problem in cattle, sheep, and wild ruminants. It also helps in describing the cytopathology observed in infected insect vectors.

Morphological features of bluetongue virus in partially purified state

Bluetongue virus morphologically, is a very complicated antigen. The capsid has 5-3-2 icosahedral symmetry displaying 96 capsomeres. The empty capsid is definitely hexagonal in shape. Four distinct phases were observed in the maturing process of the virus. It may be assumed that the first phase of maturation consists of the capsid with its capsomeres. The capsomeres are very distinct with hollow centers. In the second phase, the capsomeres have hairy projections that obscure the central core. In the third phase, the capsomeres become obliterated with a meshwork of fibrous material. The fourth phase consists of the entire particle surrounded by a unit membrane probably of cellular origin. The bare capsid is approximately 50 μ in diameter. The third phase particle is 60-70 μ , and the fourth phase ranges from 70-120 μ in diameter. The significance of this work is directed toward fractionating the complex viral particle and separating it into its component antigens. Morphologically, BTV is a very complex particle. It may occur in aggregates surrounded by a dense membrane of cellular origin. These findings shed light on the physical resistance of BTV to the destructive environments with which it may come in contact outside the host animal's body. This basic research answers some of the questions necessary for producing serological tests, identification tests, and the eventual production of an adequate vaccine against bluetongue disease of sheep and cattle. Also, it is a necessary step in determining how the gut barrier of an insect may be circumvented by an invasive virus.

Cytopathologic changes and development of inclusion bodies in cultured cells infected with bluetongue virus

Bluetongue virus caused lesions in cell cultures, including the development of 2 types of intracytoplasmic inclusion bodies. Type I inclusion bodies originated from the perinuclear space within the nuclear envelope. Material accumulated within this space and was eventually pinched off from the outer nuclear membrane, resulting in an intracytoplasmic body bounded by a single membrane. Bluetongue virus synthesis was not found associated with type I inclusion bodies. Evidence supported the theory of intranuclear origin of

type II inclusion bodies. They apparently escaped the nuclear envelope through greatly enlarged pores. Type II inclusion was ribonucleic acid (RNA)-positive in the cytoplasm and deoxyribonucleic acid (DNA)-positive in the nucleus. It fluoresced in response to specific BTV conjugate when it was intracytoplasmic; however, it did not fluoresce specifically in the nucleus. Although evidence of viral structure was not observed in the nucleus of BTV-infected cells studied in the electron microscope, mature BTV was often observed in association with type II intracytoplasmic inclusions.

Enhancement of bluetongue in sheep by previous administration of the virus

The clinical and immunologic response of sheep to a parenteral challenge BTV inoculation following a previous regimen of oral administration of the homologous virus was evaluated. The time between the onset of the oral treatment period and end challenge was most important, and an enhancing clinical response occurred when the sheep were given 4 ml. of blood virus in OCG daily for 10 days and were then given challenge inoculations on the 15th day after the start of the oral administration of the virus. The 22nd day of end challenge caused a lesser enhancing effect, while only a mild BT reaction occurred on end challenge at day 29. Mortality was increased and temperatures, leukocyte counts, mouth lesions, panting, depression, and coughing were significantly different ($p < .05$ or $< .01$) between comparable groups of sheep.

(Denver, Colorado) (ADP a3-13 and -17)

Efficacy of Commercial bluetongue vaccines for conferring resistance in sheep against challenge by bluetongue virus isolates

A commercial egg-adapted BTV vaccine and tissue culture (TC)-adapted BTV vaccine were evaluated for their efficacy to confer resistance in sheep against a challenge inoculation by 1 of 6 different BTV isolates. Both vaccines protected sheep against challenge inoculation by the homologous, virulent American standard bluetongue (BT) 8 virus. The other 5 BTV isolates caused BT clinical reactions in 50 to 100% of the vaccinated sheep upon challenge of their immunity. The numbers of sheep that had these challenge BT clinical reactions were greatly different between the 2 groups of vaccinated sheep. In the TC-adapted virus vaccine group, 8 sheep developed a typical response, 3 a moderate, and 3 a mild response to BT. In comparison, 8 out of 18 sheep from the egg-adapted virus vaccine group had BT, but these reactions were all classified as mild. However, the egg-adapted virus vaccine itself caused BT clinical reactions in 16 out of the 18 sheep vaccinated, while the TC vaccine elicited only 2 BT reactors out of the 18 sheep vaccinated. In addition, 7 of the 16 sheep that reacted to the egg-adapted virus vaccine had a BT clinical reaction typical and indistinguishable from BT produced with known virulent virus. Serial passage in sheep of the BT blood virus from 1 of the vaccinated sheep proved that the virulence of the virus was maintained.

The serum neutralization (SN) indexes of the sheep vaccinated with the egg-adapted virus vaccine were generally comparable to those obtained from sheep infected with virulent BTV. In contrast, the SN indexes of the sheep vaccinated with the TC vaccine had markedly lower indexes.

A reciprocal cross SN study on the convalescent serums from the virus control sheep indicated that 6 antigenic groups were present between the 6 virus isolates with BT OX 183 and BT 262 being similar as were BT 310, BT 318, and BT OX 193. The BT 310 antigen was not neutralized by its homologous antiserum, but the BT 310 antiserum had high protection indexes against 2 of the other virus antigens used in the study.

(Denver, Colorado) (ADP a3-14)

The direct assay for bluetongue virus by intravascular inoculation of embryonating chicken eggs

Bluetongue virus from disrupted blood or ground insects may be directly assayed for concentration by intravascular inoculation of embryonating chicken eggs. Intravascular inoculation of egg-adapted virus enhanced the virus titer on the average of the order of 2 logs and gave more reproducible results than did injection by the yolk sac. Tissue culture-adapted virus may be titrated by intravascular inoculation into embryonating chicken eggs. A modified technique utilizing instrumentation was developed which allowed one person to rapidly and accurately inoculate large numbers of eggs by the intravascular route.

(Denver, Colorado) (ADP a3-15)

Transmission of attenuated and virulent bluetongue virus with Culicoides variipennis infected orally via sheep

Attenuated bluetongue virus (commercial vaccine, chicken embryo origin) was transmitted from vaccinated to normal sheep by the bite of the small blood-sucking fly Culicoides variipennis. Serial transmission of the vaccine virus from sheep to sheep was also shown, i.e., the recipient host of one test served as the infecting donor of the next test, and so forth. Virus isolation studies showed that the bite of a single infected fly transmitted BT. Of the 14 sheep used either as infecting donors, recipient hosts, or both, 10 had typical experimental signs of BT. The percentage of all flies harboring the vaccine virus after feeding on infected donor sheep was 6%. Comparable virus transmission and isolation tests involving the virulent virus were also conducted.

(Denver, Colorado) (ADP a3-16)

B. Vibriosis in Sheep

Research under a cooperative agreement with the Colorado Agricultural Experiment Station was continued. Investigations were continued to determine the duration of immunity in ewes vaccinated in 1963 with Vibrio fetus serotype I and V oil adjuvant bivalent bacterin. These ewes were subsequently given challenge exposure orally during advanced gestation at 2, 3, 4, 5, and 6 years of age, with the combined V. fetus serotype I and V organism. Immunity challenge was made each year in nonexposed designated lots of vaccinated and nonvaccinated ewes.

In 1966-67, the immunity of 57 five-year-old pregnant ewes, arranged in lots 10, 11, and 12 was challenged with the following results: Lot 10, 12 ewes, nonvaccinated, challenged - 5 vibronic abortions; lot 11, 22 ewes, vaccinated, challenged - 1 vibronic abortion; lot 12, 23 ewes, nonvaccinated, nonchallenged controls - 1 full-term lamb dead from an undetermined cause.

From data obtained in the 1967 duration of immunity studies, 5-year-old ewes vaccinated when yearlings, had strong immunity against oral challenge with the combined V. fetus serotype I and V organisms given during advanced gestation of their fourth pregnancy.

(Fort Collins, Colorado) (ADP a3-11)

In cooperation with the Montana Agricultural Experiment Station, investigations were continued on ovine vibriosis.

Attempts to develop an effective selective medium for the isolation of V. fetus from contaminated materials have not succeeded. Three antibiotics commonly used in the isolation of V. fetus were used in trials, which proved that they are ineffective against common contaminants at levels and in combinations that do not inhibit V. fetus. At levels effective against contaminants, they inhibit the growth of V. fetus.

Bacteriophage was isolated from 5 lysogenic cultures of V. fetus. Insofar as is known, phage has not previously been isolated from V. fetus. The phages isolated are being characterized.

The serologic and physiologic characteristics of 62 Vibrio fetus isolated was completed. There are 3 somatic serotypes of V. fetus and at least 7 heat-labile antigens.

(Bozeman, Montana) (ADP a3-11)

For the 7th consecutive year, the effect of vaccination of yearling replacement ewes with commercial V. fetus vaccine containing Serotypes I and V organisms to prevent vibriosis was studied in cooperative research at the Utah Agricultural Experiment Station. There were 2 herds with approximately

2000 ewes each. In herd A, one V. fetus isolant was recovered from 23 aborted or stillborn lambs and 3 V. fetus isolants were recovered from 105 placentas taken from ewes having apparently normal parturitions. In herd B, V. fetus was not isolated from 49 aborted lambs, but was recovered from 3 placentas out of 101.

All the ewes in herd A were also vaccinated for enzootic abortion of ewes for the 2nd consecutive year while those in herd B were not. The number of aborted or stillborn lambs infected with psittacosis-lymphogranuloma virus (PLV) was less in herd A. In herd A, 13% were infected and 22% in herd B. In both herds, 8% of placentas were PLV-infected. There was a reduction of infection in herd A as compared to previous years. We need to clarify whether or not this reduction is the result of the 2 years' vaccination.

The yearly use of a commercial ovine vibriosis bacterin on replacement ewes, when injected twice, 3 weeks apart, has continued to control vibrionic abortion in 2 herds. A residual infection or reinfection can exist in a herd but the yearly vaccination of susceptible replacement ewes can keep losses at a minimum.

(Logan, Utah) (ADP a3-11)

C. Paratuberculosis of Sheep

The study on the pathogenesis of paratuberculosis in sheep was continued. Preliminary information was obtained on the rate at which Mycobacterium paratuberculosis invades certain tissues.

(Ames, Iowa) (ADP a3-6)

D. Ulcerative Dermatitis of Sheep

In cooperative research with the Colorado Agricultural Experiment Station, investigations are being conducted with the objectives of experimentally reproducing ulcerative dermatitis and identifying the causative agent(s).

Clinically, ulcerative dermatosis has been recognized as a disease entity of sheep. The disease will affect often more than 30% of the flock. Ulcerative lesions are encountered on the genital organs, feet, face, and eyes. The genital form deters breeding and may result in a reduced rate of conception, and consequently smaller lamb crop. Lameness in the pedal form deters breeding by rams and causes deterioration of physical condition and loss of body weight.

For reproduction of the disease, infectious materials were obtained from field cases which involved hundreds of sheep in 3 different areas in Colorado. The disease could be reproduced in experimental sheep; however, if bacterial isolates were used alone they would not produce disease.

Isolation and identification of causative agent(s) -- both bacterial and viral in nature -- were investigated. Tissue culture and chicken embryo techniques were employed. No significant agent has been found. Investigation is being continued.

(Fort Collins, Colorado) (ADP a3-4 (R))

E. Toxicological Effects of Oxalate-Containing Plants

Lethal amounts of Halogeton glomeratus (oxalate) were fed to sheep. These sheep developed a hypocalcemia, hypermagnesemia, and hyperphosphatemia. There was an increase in serum lactic dehydrogenase, serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase. These increases were thought to reflect the marked tissue damage to the rumen and kidney. The possibility of damage to the myocardium was suggested.

Tissue succinic dehydrogenase was inhibited in rumen tissue. This fact suggests the possibility of an interference of carbohydrate metabolism in halogeton poisoning.

There is good evidence that there are factors other than hypocalcemia in death of sheep from halogeton poisoning.

(Logan, Utah) (ADP a3-7 (R))

F. Clinicopathologic and Preventative Aspects of Poisonous Plants on Livestock

Ingestion of Veratrum californicum as a cause for dry ewes

The number of dry ewes (nonpregnant ewes at lambing time) has decreased from 10 to 15% to 1 to 1.5% since the ewes have been prevented from grazing Veratrum-infested range areas during the breeding season. The incidence of the congenital cyclopic deformities in lambs always paralleled the incidence of "dry ewes." The annual economical loss to sheepmen has been estimated at \$1,000,000 a year. The "dry ewes" would be sold as culls at the end of the lambing season for \$2.50 to \$5.00 per head while it would cost from \$20 to \$28 a head to replace each "dry ewe."

The research studies in determining that ingestion of Veratrum californicum was the cause of congenital deformities in lambs and dry ewes has been very beneficial in helping many sheepmen reduce their annual loss and enabling them to remain in the sheep-raising industry.

Veratrum californicum may stop growth of the leg bones in the fetus

Veratrum californicum causes a cessation in the growth of fetal leg bones when maternally ingested from the 30th to 35th day of gestation. This plant

contains many toxic agents, but further study is necessary to determine its many possible effects on animals.

A standard for determining age of fetus by measurement

A standard procedure for measuring the body and limbs of a lamb fetus has been established with the weights and measurements of individual bones in cooperation with Dr. Howard Evans, Anatomy Department, New York State Veterinary College, Cornell University. This information is very important in determining the age of fetuses. The information can be made available for all those who are interested.

Two species of lupine, cause a congenital deformity in calves (*Lupinus sericeus* and *Lupinus caudatus*)

A congenital deformity called the "crooked calf disease" has occurred in many western beef-producing areas. The deformity has only been observed in calves whose dam was grazing lupine-infested range areas during the early stages of gestation. The deformity is characterized by twisted legs with contracted joints, twisting or contraction, or both, of the cervical vertebra and twisting of the back and cleft palate. All of the above deformities may occur in the same animal or just one, depending on the time of insult to the fetus. The deformity is more severe in the front legs when the lupine is ingested between the 40th and 70th day of gestation. The deformity is nonhereditary and is associated directly with grazing range areas.

Prevention of the congenital deformity has been accomplished by individual ranchers by delaying the breeding season for 30 days or by supplementing the animals throughout the grazing season with a phosphorus supplement.

(Logan, Utah) (ADP a3-18)

G. Scrapie

Scrapie was first diagnosed in the United States several years ago. It is, however, not firmly established and efforts are continuing to eradicate it. Research has been conducted on this disease in Scotland and Great Britain since 1961. The USDA is supporting this research through P.L. 480 grants. In recent years, it has been determined that the disease is probably caused by a transmissible agent. The agent has, however, not been isolated nor characterized in detail. There is also increasing evidence that a certain genetic constitution is existent which determines susceptibility.

(Scotland E29-ADP-4) (England E29-ADP-5)

H. Pulmonary adenomatosis

The views of various investigators regarding the inflammatory or neoplastic nature of sheep pulmonary adenomatosis are controversial. Reviewers of the problem have regarded the disease as occurring in a variety of forms, ranging from infiltrative pneumonia to metastasizing carcinoma. Many have considered that the condition constituted a complex of related diseases and hence have referred to them as the sheep pulmonary adenomatosis complex. Research was initiated during the year at the College of Veterinary Science and Animal Husbandry, Mathura, Uttar Pradesh, India through the support of a P.L. 480 Grant.

(India A7-ADP-23)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Bluetongue

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Scrapie

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MacKenzie, A., and Wilson, A. M. 1966. Accumulations of fat in the brains of mice affected with scrapie. Res. Vet. Sci. 7:45-54.

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AREA NO. 4 - DISEASES AND PARASITES OF HORSES

Problem. Currently there are about 3,250,000 horses in the United States, valued at approximately \$860 million. About 1 million of these are draft animals. Considerable numbers of horses and mules are still required for work on cattle ranches and as pack animals. The annual overall value of the horse industry has been estimated at about \$1.5 billion. The horse may be an important link in epizootiology of animal diseases in general. Equine piroplasmosis is an acute, subacute, or chronic tick-borne disease of horses caused by protozoan parasites that was first recognized in this country in Florida in 1961. It is characterized by high fever, progressive anemia, jaundice, edema, extreme weakness, and depression. Fatalities range from 5 to 50% of infected animals. This disease, now apparently well established in Florida, has extended into Georgia and poses a serious threat to the entire equine population in the southern United States. The disease is clinically indistinguishable from equine infectious anemia. Horses that have clinically recovered from piroplasmosis usually remain carriers of the disease and are a potential source of infection. African horsesickness, a highly fatal disease of equine animals that was confined to Africa, has caused serious losses in the Middle East and parts of Asia in recent years.

USDA AND COOPERATIVE PROGRAM

The Department has a program involving biochemists, pathologists, protozoologists, and veterinarians working on equine piroplasmosis. In order to be prepared in the event of introduction of African horsesickness into the United States, the Plum Island Animal Disease Laboratory has obtained African horsesickness viruses and antiserums from South Africa. These materials are thus directly available for diagnostic and vaccine studies should the need arise.

The Federal scientific effort devoted to research in this area is 6.0 scientist man-years. This effort is divided among subheadings as follows:

Serological Diagnosis, Transmission, and Control of Equine Piroplasmosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland (in cooperation with the Entomology Research Division), and under contracts with the University of Florida, Gainesville, and the University of Kentucky, Lexington.

Equine Infectious Anemia 4.0 at the Washington State University, Pullman, the Texas Agricultural Experiment Station, College Station, the Louisiana State University, Baton Rouge, and at the National Animal Disease Laboratory, Ames, Iowa.

P.L. 480 funds have been made available in Turkey for research on Gastrophilus pseudo-haemorrhoidalis (equine parasite) in Turkey, and for the study of the horsesickness virus.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 12.5 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Equine Piroplasmosis

Investigations on the serological diagnosis, transmission, and control of this disease are being continued.

Developmental cycles have been described for Babesia caballi, and for B. equi in the vertebrate host. The developmental cycle of B. caballi in the tropical horse tick, Dermacentor nitens, has been studied and described. The parasite has a deleterious effect on the reproductive potential of its vector. Normal ticks have been infected with B. caballi by feeding on blood from donor horses infected by Babesia-carrying ticks. An indirect fluorescent antibody test for B. caballi has been developed, but some modifications may be required before it is fully acceptable.

(Beltsville, Maryland) (ADP b6-13)

Research studies under a contract with the University of Florida were initiated. They are aimed at identifying drugs useful in preventing, treating and eradicating piroplasmosis in horses.

A total of 16 horses were infected with Babesia equi. Three were intact horses while the remaining 11 were splenectomized. Signs of infection were not severe in intact horses but were acute in the splenectomized ones.

Eight of the 11 splenectomized horses died from acute babesiosis. Two were treated with Camolar, 1 with Diaminostibene, 3 with Phenamidine and 2 with Berenil. The first 3 horses were treated as soon as organisms were detected but without effect on the clinical signs. Approximately 10% of the erythrocytes were infected at the time the first doses of Phenamidine and Berenil were given but the signs progressed in the usual manner.

The 3 splenectomized horses, which survived the infection, were treated when the 1st organisms were demonstrated in the erythrocytes. One was given Phenamidine at the rate of 4 mg./lb. of body weight on each of 2 consecutive days. A rise in body temperature was present for 2 days but signs were never severe. Another horse was given Phenamidine at the rate of 5 mg./lb.

of body weight on 3 consecutive days. It had severe signs for 4 days but no permanent damage can be detected.

One horse was treated with Diampron, 5 mg./lb. of body weight on 4 consecutive days. Clinical signs were present for several days but it made a complete recovery. The 3 horses are under test to determine whether the "carrier" state was eliminated by the treatments.

The 3 intact horses were treated with Phenamidine. One was given the drug on 4 consecutive days and the other 2 were given the drug on 4 consecutive days. The dosage was 5 mg./lb. of body weight. The first horse had toxic signs on the 4th day and treatment was discontinued. All were free of infection when blood was serially passaged. They are being held for another blood transfer at a later date.

Enzyme and electrolyte determinations are being made on serums of infected and treated animals. Insufficient determinations have been made for accurate evaluation of the results.

Studies are in progress to determine the time required for B. caballi to be removed from infected horses. Horses that were given challenge exposure did not have clinical signs of infection after being given blood from treated horses.

Blood transfers have been completed to determine the status of the original infections of B. caballi and B. equi established at the laboratory. Serums are being prepared for complement fixation tests. Test results will help in evaluating the test and determining the status of the infection.

(Gainesville, Florida) (ADP b6-13(C))

In the cooperative research at the Kentucky Agricultural Experiment Station, the study was concerned with the histopathological changes which occurred in a serial sacrifice of 9 splenectomized ponies inoculated with B. caballi. In each pony in the serial sacrifice experiment, the primary damage occurred in the lungs, liver, kidney, and heart. The extensive mononuclear cell infiltration was a constant finding together with changes associated with blood destruction.

The histopathologic lesions were quite consistent in the ponies and, after the first 3 were euthanatized, the remaining ponies had almost identical signs and gross and microscopic lesions.

The myocardial lesions were interesting. Apparently, heart damage contributed to death, and anemia was not the apparent cause. The microhematocrit reading had an appreciable drop in only 2 ponies. One had a greatly increased microhematocrit reading and died before euthanasia.

Pathological changes in the lungs were progressively worse. There was a sequestering or round cells in the arterial branches with resultant round cell infiltration that caused a broadening or thickening of the alveolar walls.

The microscopic changes in the liver of these ponies with B. caballi and in those with equine infectious anemia are almost identical. However, in the absence of detectable intra-erythrocytic organisms, differentiation would be most difficult.

The kidney damage was about the same in the latter part of the series. This damage was not severe in any of the ponies.

(Lexington, Kentucky) (ADP b6-13(C))

B. Equine Infectious Anemia

Emphasis has been placed on the development and evaluation of diagnostic tests. The complement fixation test, hepatic biopsies, siderocyte counts, and serum protein alterations were evaluated in 30 horses experimentally infected with the virus of equine infectious anemia (EIA). The test procedures were carried out sequentially to determine their usefulness as specific diagnostic criteria or as laboratory aids in the diagnosis of EIA. The complement fixation antibody titer usually reached its highest levels during or shortly after the first fever elevation, then slowly decreased over a variable period of time to become undetectable. Subsequent febrile episodes did not result in a recall response. Siderocyte counts occurred in varying numbers in all horses with active disease. Siderocytes were usually demonstrated in the circulation of 2 horses that had been afebrile for about a year, but sometimes they were absent. Morphologic hepatic lesions of EIA occurred in horses during or shortly after the first fever and in horses with active disease. These changes persisted more than 9 weeks after severely affected horses had experienced their last febrile episode. The percentage of gamma globulin in serum increased and the albumin/globulin ratios decreased in most infected horses. The levels of these alterations varied somewhat in individual horses and between horses.

(Pullman, Washington) (ADP a4-2(CA))

At the Texas Agricultural Experiment Station the nature of the research was very basic and evolved around characterization of the virus and its effect upon the animal. These basic questions must be answered before any practical progress can be made on control of the disease.

The virus of equine infectious anemia was adapted to grow in horse leukocyte cultures. Growth of the virus in these cultures caused horse red blood cells to be attracted to the leukocyte cells in culture, which provided a quick reliable means of evaluating the effect of chemical agents upon the virus. In this method, ether completely disintegrated the virus particle.

The virus of equine infectious anemia induces certain cells in the body to produce a protein that is excreted in the urine. This material also occurs in the leukocyte cultures as well as in the blood stream. In all probability, this material and the antibody to the protein cause the lesions seen in the horse.

With the growth of the virus in tissue culture, separation and purification of the virus became a reality. With a purified virus fraction, specific antiserum could be made to the virus for the 1st time. This antiserum was used to determine (1) if antibody was produced to the virus in the horse, (2) to produce an experimental fluorescent antibody test, and (3) to determine the site and greatest concentration of virus in the body. Antibody to the abnormal protein was also produced and used in the same manner. These procedures are necessary so that information on the virus-animal interaction may be obtained. This information is vital before any procedure such as production of a vaccine or control measures could be instituted.

(College Station, Texas) (ADP a4-2(CA))

In cooperative research at the Louisiana State University, Baton Rouge, the results of studies on the precipitin test indicate that both false positives and false negatives occur. This conclusion is based upon the comparative results of horse inoculation tests and by challenge exposure to known equine infectious anemia virus.

The Shetland pony was highly susceptible to the Wyoming strain of equine infectious anemia virus. Seven of 8 ponies died or were killed when it was thought that death was imminent.

The oral transmission of equine infectious anemia virus to a 1-day-old Shetland colt was accomplished. Following severe illness characterized by fever, weakness, and bloody diarrhea, the colt died on the 17th day after exposure.

(Baton Rouge, Louisiana) (ADP a4-2(CA))

C. Gastrophilus Pseudo-haemorrhoidalis

In Turkey, under a P.L. 480 Grant, a survey has been made for Gastrophilus pseudo-haemorrhoidalis of horses from 1962 to 1967. A total of 6295 equines

(5112 horses, 1134 donkeys, and 49 mules) were examined for this parasite and 14,741 larvae of different *Gastrophilus* species were obtained from 2111 infested equines. Of these, 129 larvae had the morphological characteristics of *G. pseudo-haemorrhoidalis*. Nineteen of 94 larvae placed in insect-proof cages hatched into flies, but only 3 (1 male and 2 females) reached full maturity. It was clear that the morphological characteristics of these flies were not different from those of *G. haemorrhoidalis*. The significant facts concerning their life cycle and different habits could not be determined because they died 30 hours after they became mature.

During the period of this report, 1779 adult flies of *Gastrophilus* were captured in pastures and 61,454 eggs collected from the coats of many equines. They all represented *G. intestinalis*, *G. nasalis*, *G. haemorrhoidalis*, *G. inermis*, *G. pecorum*, and *G. meridionalis*.

The effect of Neguvon against bots was tested on 2019 equines (1808 horses, 197 donkeys, and 14 mules). Neguvon was administered to each at the rate of 20, 25, 30, 35, and 40 mg./kg. in feed. The experiment showed that Neguvon was highly effective against bots when used orally at the rate of 30, 35, and 40 mg./kg. It did not give good results at 20 and 25 mg./kg. The larvae attached on the wall of the rectum were not affected in most of the animals by Neguvon applied orally at the dose rates mentioned. The treated animals expelled both live and dead larvae in their feces. Some of the live larvae collected from feces of treated animals hatched into adult flies. Undesirable effects of Neguvon were not seen, even in pregnant animals. Larvae appeared in the feces 2 hours after Neguvon administration and lasted 144 hours.

(Ankara, Turkey) (A22-ADP-7)

D. African Horsesickness Virus

Under a P.L. 480 Grant to the Veterinary Faculty, University of Ankara, Ankara, Turkey, studies have continued on the cultivation of the virus in tissue culture, its serological and immunological characteristics, as well as localization site and duration of the virus in the body of the vectors.

Vaccine strains of mouse-adapted African horsesickness virus killed mice in 5 to 7 days when given intracerebrally. Mice were also killed in 5 to 7 days when given inoculum of the same virus passaged 15 times in mouse and hamster kidney cells. After 15 passages, the killing time was lengthened and mice were killed in 7 to 9 days at the 20th passage.

Beginning with the 10th tissue culture passage, 1 horse was given 5 cc. subcutaneously and 1 was given 10 cc. intravenously. The horses were then observed for a month and had no signs of African horsesickness. However,

1 horse inoculated intravenously with 10 cc. from the 20th passage died after 44 days with no signs of horsesickness. Necropsy revealed edema of the orbital area and under the skin of the lips. Specimens of liver, spleen, lung, and blood were taken and inoculated in tissue culture and mice. The inoculum did not produce a cytopathogenic effect in tissue culture nor kill the mice. Therefore, it was concluded that this horse did not die of African horsesickness.

Neutralization tests were made in mice and tissue cultures to detect antibodies in the blood of 2 horses given 10 cc. of tissue culture virus intravenously. Antibodies were not detected at 5 and 10 days after virus inoculation. Blood samples taken at 15, 20, and 30 days after virus inoculation contained 5 x LD₅₀ virus neutralizing antibodies.

The blood of horses inoculated subcutaneously with 5 cc. of tissue culture virus was tested for antibodies on 5, 10, 15, 20, and 30 days after inoculation. At 10 days after inoculation, 10 x LD₅₀ virus neutralizing antibodies were established.

(Ankara, Turkey) (A22-ADP-7)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Equine Piroplasmosis

Holbrook, A. A. 1965. Equine piroplasmosis and its diagnosis. Proc. 11th Ann. Conf. Am. A. of Equine Prac., 157-166.

Equine Infectious Anemia

Moore, R. W., Redmond, H. E., and Lewis, D. N. 1967. Equine infectious anemia. A diagnostic problem. Proc. 69th Ann. Meet. U. S. Livestock San. A. 255-260.

_____. 1967. Equine infectious anemia, recent research findings. Proc. 1st Internat. Conf. on Infect. Dis. of the Equine.

AREA NO. 5 - INFECTIOUS AND NONINFECTIOUS DISEASES OF POULTRY

Problem. Annual losses from infectious and noninfectious diseases of poultry, exclusive of parasitisms, are estimated to be at least \$200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production, and lowered quality, are the final losses occasioned by condemnations at dressing plants. Turkey growers are still faced with the problem of widespread Mycoplasma infection in flocks throughout the country. Condemnation losses from Marek's disease in broilers seem to be increasing. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA AND COOPERATIVE PROGRAMS

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and noninfectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 16.5 scientist man-years. This effort is applied as follows:

Ornithosis 2.7 at the National Animal Disease Laboratory, Ames, Iowa.

Salmonellosis 1.0 at the Southeast Poultry Research Laboratory, Athens, Georgia.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 5.3 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Georgia, and Wisconsin, and with the University of Minnesota.

Newcastle Disease 3.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station, and under a P.L. 480 Grant to the Institute for Veterinary Research, Pulawy, Poland.

Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

Infectious Bronchitis 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 79.2 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chronic Respiratory Disease Complex

A study was conducted at the Southeast Poultry Research Laboratory, Athens, Georgia, on the effect of various temperatures on fertility and hatchability of normal and Mycoplasma gallisepticum-infected chicken eggs.

Normal White Leghorn chicken hatching eggs were subjected to pre-incubation heat treatments at 110 to 116 F. for 4 to 8 hours. Eggs heated at 116 F. for 6 hours maintained approximately 25-40% fertility and only 5-15% hatchability. However, at 114 F. for 6 hours, the eggs had approximately 55% fertility and 20-30% hatchability. Higher temperatures or longer treatments were more drastic.

Similar pre-incubation heat treatment of eggs from chickens following artificial infection was studied as a means of inactivating M. gallisepticum within the test eggs. No isolates of M. gallisepticum were obtained from any of the eggs which were heated at specific temperatures between 114.1 and 116.4 F. for 6-hour periods. A total of 38 unheated control eggs contained viable M. gallisepticum. Current studies suggest that the minimum temperature for inactivation of M. gallisepticum during the 6-hour pre-incubation period is somewhere between 113 and 114 F.

Various combinations of ingredients were studied to devise more adequate mediums for the isolation and cultivation of avian Mycoplasma. Tryptose phosphate broth was most adequate when supplemented with 15% swine serum, 0.5% Difco proteose peptone #3 and 0.5% Albimi yeast autolysate. Similar medium with horse serum was only slightly less adequate. However, freshly prepared turkey meat infusion broth with 15% turkey serum and 5% homemade yeast autolysate was superior for the isolation of M. gallisepticum from infected hatching eggs.

(Athens, Georgia) (ADP a5-29)

At the South Central Poultry Research Laboratory, State College, Mississippi, investigations were conducted on the effect of modified environments on Mycoplasma infections in chickens.

The study revealed that chickens given pathogenic Mycoplasma gallisepticum intranasally at 6 weeks of age were shedding the organisms from 29 to 43 weeks of age as evidenced by tracheal isolations and spread to susceptible control birds. The progeny of the exposed birds were not free of M. gallisepticum since isolations were made from 18-day embryonated eggs and from 1-day-old chicks.

A culture medium for the production of M. gallisepticum antigen was developed that would produce from 12.0-18.0% yield of serum plate antigen when concentrated 2 x No. 10 McFarland nephelometer. The medium that produced optimal growth was 3.7% brain heart infusion, 0.5% yeast autolysate, 0.0005% thiamin hydrochloride, 0.3% dextrose, 0.3% Tris buffer, 0.05% trypticase, 10% inactivated swine serum, 90% distilled deionized water, with 0.02% thallium acetate and 200 units of penicillin per ml. to combat contamination.

The results of 2 experiments show that a broiler chick can withstand low temperatures (4.4 C.) for short periods of time (12 hours) without significantly affecting 8-week body weight, mortality, and condemnations.

Results of experiments designed to study the effect of temperature and density on broiler performance showed a significant density effect on body weight when broilers were reared the last 5 weeks at temperatures below 21.1 C. With temperatures between 21.1 C. and 37.8 C., the density effect on body weight is eliminated at the 5% level of probability when density levels of 650 and 929 square centimeters per bird were compared. Mortality and condemnation rates were not significantly affected by temperature and density; however, there was a better feed conversion at the higher temperature level.

(State College, Mississippi) (ADP a5-36)

In cooperative work at the Agricultural Experiment Station of Georgia, the activities have been centered on details of methodology which would afford a better quality fluorescent antibody conjugate and specimen for observation.

The use of these techniques in the detection of Mycoplasma gallisepticum in clinical material is a feasible tool in competent hands. It is strongly recommended that a chelated azo dye such as eriochrome black be used as a counterstain in this procedure to eliminate undesirable nonspecific fluorescence. Characterization of observed fluorescent organisms as members of the S-6 serogroup can presumptively be made at the time of observation. The use of growth inhibition in the presence of high titered antiserum has proved valuable in the characterization of cultured isolates.

(Athens, Georgia) (ADP a5-29)

At the North Carolina Agricultural Experiment Station an evaluation was made on the effects of Mycoplasma gallisepticum and the infectious bronchitis virus on the susceptibility of chickens to infection with Escherichia coli (E. coli).

It has been established that vital organs such as liver, spleen, and lungs are important in the removal of E. coli organisms from the circulating blood. Infectious bronchitis virus is capable of reducing this efficiency.

The importance of developing M. gallisepticum-negative breeding stocks has been further substantiated by comparing the performance of broilers from Mycoplasma-negative parents with that of naturally-infected as well as experimentally-infected parents.

As the result of these findings, an official eradication program has been jointly adopted by the broiler industry and regulatory officials in North Carolina.

(Raleigh, North Carolina) (ADP a5-29)

In cooperative work at the University of Minnesota, epidemiological investigations were conducted on all known and suspected outbreaks of infectious sinusitis during the year. Roughly 95,000 grower turkeys and 3,500 breeder hens were found to be infected with the disease during this period. One breeder flock was exposed to contaminated produce trucks and the other flock's source of exposure was undetermined. Over 42,000 eggs were destroyed. All growing birds were exposed to infected poultry. More emphasis on the part of the turkey industry is being placed on complete separation of breeder and growing operations to avoid contact exposure. No contaminated vaccines were encountered this past year.

Ornithosis continues to be constantly present in turkey raising areas. Tests on 2,806 serum samples from 90 flocks in Minnesota, Iowa, Wisconsin, and other states were conducted for ornithosis. Six positive flocks were found in Minnesota, 1 in Wisconsin, 1 in Iowa and 2 in California. No virus isolations were made and no cases in human beings were reported resulting from contact with infected flocks.

The cycle of Mycoplasma meleagridis infection has been worked out. The disease is primarily a reproductive disease in both the male and female. The infected male eliminates the organism in the semen and the infection becomes established in the reproductive tract of the female. The organism infects the egg in the lower part of the reproductive tract. It becomes established in the anterior air sacs of the embryo producing lesions in the poult at the time of hatching. It is widely distributed in turkey breeding flocks.

A pilot control program has been proposed for M. meleagridis. The program involves the primary breeding stock. The first step in the program is dipping the hatching eggs in tylosin and selection of the eggs early in the laying cycle. The eggs must be hatched in an incubator isolated from undipped eggs. The progeny must be raised isolated from poults from undipped source. The toms used for mating the progeny must be tested for M. meleagridis. If negative toms are available, then only these toms will be mated with the females. Eggs from this mating will be dipped in tylosin. The male and female populations will be tested with M. meleagridis antigen to determine their status. If serologically negative, the eggs do not have to be dipped. If serologically positive, negative males are selected on the basis of examination of the semen.

A Mycoplasma-free commercial type breeder flock was obtained by egg dipping, selection of eggs early in the laying cycle, and mating flock with toms not shedding the organism in the semen.

(St. Paul, Minnesota) (ADP a5-21)

In the cooperative research at the University of Wisconsin Agricultural Experiment Station, the following work was accomplished:

Determination of host parasite relationships - Nature of disease

Since the time of the last annual report (June 30, 1966) the 14th and 15th consecutive turkey flocks have been raised in the Meteoropathology Building.

Under the conditions of this study, atmospheric dust significantly increased the incidence of airsac lesions in turkeys.

Ammonia did not have a significant effect on the incidence of airsac lesions in turkeys. No significant interaction between ammonia and dust concentrations affected the incidence of airsac lesions. Also, no significant effects of dust and ammonia upon mortality were detected. Prolonged exposure (10 wk.) to concentrations of ammonia ranging from 20 to 50 p.p.m. resulted in some loss of cilia from the columnar cells lining the lumen of the trachea.

Studies on isolation and characterization of the causative agents resulted in the following:

A⁺ human plasma incorporated into Albimi-French (AF) PPLO broth medium gave better results than horse serum for initial isolation and continued propagation of Mycoplasma meleagridis.

Mycoplasma meleagridis had a faster growth rate and higher end-point titer in chick embryo fibroblast (CEF) or chicken kidney (CK) cell cultures than in the broth medium or in embryonating hens' eggs.

No cytopathic effects were seen in CEF or CK cell cultures following inoculation and growth of M. meleagridis.

(Madison, Wisconsin) (ADP a5-21)

Under a P.L. 480 Grant to the Hebrew University, Hadassah Medical School, Jerusalem, Israel, research on the structure, chemical composition, immunochemistry and nutritional requirements of PPLO (Mycoplasma) pathogenic to farm animals has produced the following results:

The development of methods for the separation of Mycoplasma membrane proteins accomplished during the last year has enabled the partial biophysical and immunological characterization of these proteins. Although this field of study is still at an early stage, it already produced a very useful method for the identification of Mycoplasma strains by the electrophoretic patterns of their membrane proteins. There are some indications that this method can be applied to whole cells, simplifying the procedure considerably and enabling its wide use. The immunological characterization of membrane proteins is still impeded by the difficulty of solubilizing these hydrophobic proteins without affecting their antigenicity. However, the results obtained so far are encouraging by showing that these proteins dissolved in sodium dodecyl sulfate (SDS) still keep at least part of their serological activity.

No definite conclusion may be drawn as yet whether Mycoplasma membranes are built of lipoprotein subunits or of alternating leaflets of protein and lipid. The results obtained so far with M. laidlawii membranes indicate that membrane lipid is closely associated with membrane protein in the SDS disaggregated material, supporting the subunit hypothesis. The extension of this study to membranes of M. gallisepticum and bacterial L-forms, the use of electron microscopy and the quantitative study of the membrane reaggregation phenomenon will probably help to elucidate this most important and basic problem of membrane structure.

(Jerusalem, Israel) (A10-ADP-9)

B. Salmonellosis

At the Southeast Poultry Research Laboratory, Athens, Georgia, further study was conducted on salmonellosis and the related enteric infections:

Polystyrene latex particles sensitized with the somatic antigens of 3 strains of Salmonella typhimurium have been successfully utilized to prepare agglutination antigens for use in the rapid whole-blood, serum plate, and tube agglutination tests. Indications are that latex particle antigens are more sensitive to S. typhimurium agglutinins in naturally infected flocks than are conventional tube agglutination antigens prepared from

S. typhimurium, strain P-10. Antigenic materials representative of more than 1 Salmonella group may be adsorbed on latex particles to provide a polyvalent type antigen. A combination antigen containing S. typhimurium and S. pullorum is being investigated initially in this regard.

Preincubation fumigation of chicken eggs with a high level of formaldehyde gas (1.2 ml. CH₂O per cubic foot) has revealed that statistically there is no significant effect on hatchability of eggs set following fumigation. This level of the fumigant was effective in the complete destruction of Salmonella organisms deposited on the surface of the egg shell except when abnormally high populations of the organisms were used for contamination prior to fumigation. Levels of formaldehyde are higher on the surface of brown eggs than on the surface of white ones following fumigation.

Procedures for studying Salmonella penetration through the 3 outer areas (shell, outer membrane, inner membrane) of chicken eggs have been further perfected to the degree that a single egg can be sampled in the above 3 areas in about 1 minute. Hatching eggs are more resistant to Salmonella penetration as they are incubated and embryonic development takes place. Penetration studies with cracked eggs showed that 100% of cracked eggs are penetrated in all 3 outer areas after 24 hours' incubation compared to only 40% penetration of control groups. Work is being directed toward the development of a chemical protection against shell penetration for the full 21-day incubation period.

(Athens, Georgia) (ADP a5-30)

To determine the cycle of Salmonella infections in turkeys, cooperative research was conducted at the University of Minnesota using 2 Salmonella serotypes, S. heidelberg and S. saint paul.

The experiences of Hatchery A indicated that even though S. heidelberg may exist at a low level in the breeding flocks, the organism will cycle through the hatchery and infect the progeny. The mortality may remain low but total mortality to 10 days in approximately 110,000 poults on 3 farms was 3.8%. Premise contamination may share as a source of Salmonella infections as evidenced by the recovery of S. senftenberg, S. heidelberg, and S. tennessee from the environment on the 3 farms.

The monitoring of Salmonella infections in Hatchery B revealed that culturing of dead embryos was a more sensitive means of determining the presence of Salmonella and Arizona organisms than the vent squeezings at the time of sexing of day old poults. It emphasized the higher rate of egg transmission of Arizona organisms as compared to S. saint paul.

In cooperation with the Minnesota Livestock Sanitary Board (M.L.S.B.) and the Salmonella testing laboratories, turkey breeder flocks found infected with S. saint paul, and S. heidelberg were available for studies.

Approximately 500 flocks involving 650,000 birds are under supervision of the M.L.S.B. They are tested with pullorum and typhimurium antigens. Reactors to the test are submitted for bacteriological examination and Salmonella and Arizona isolates are sent for serotyping. Reactors to the serological tests were in 221 flocks but on bacteriological examination of selected reactors from each flock, 67 flocks were infected. Twelve different serotypes were identified. S. saint paul was isolated from 23 flocks, S. typhimurium from 3 flocks and S. heidelberg from 1 flock.

The 3 flocks infected with S. typhimurium were approximately 300 miles from the laboratory and were not included in the study because of the difficulty in monitoring the flocks. The 3 flocks were retested with typhimurium antigen and were negative serologically on 2 retests.

(St. Paul, Minnesota) (ADP a5-33, (CA))

C. Pasteurellosis

At the National Animal Disease Laboratory, Ames, Iowa comparisons were made on the survival of Pasteurella multocida cultures after shell-freezing, freeze-drying, and maintenance on agar slants at various temperatures. Pasteurella multocida suspended in a mixture of tryptose broth and skim milk and shell-frozen in a concentration of 4.45×10^8 colony-forming units (CFU) per ampule resulted in a survival of 58%. After the shell-frozen cultures were stored for 3 to 12 months at -62 C., an average survival of 47% and 43% CFU, respectively, was obtained. Cultures freeze-dried from the shell-frozen state at 4 different pressure levels and 6 drying times, following immediate reconstitution, resulted in an average survival of 21.2% CFU. After the freeze-dried cultures were stored for 3 and 12 months at 5 C., survival was reduced to an average of 12.5% and 0.6% CFU, respectively. Agar slant cultures qualitatively inoculated with Past. multocida were viable after 7 months of storage at 25, 5, -23, and -62 C. No alterations in colonial or biochemical characteristics were noted for Past. multocida cells which were shell-frozen or freeze-dried or those maintained on agar slants at 5, -23, and -62 C. However, after 2 months of storage at 25 C., agar slant cultures were observed to dissociate and provide a mixture of fluorescent, blue, and sectorial colonies.

A heat-stable, particulate, lipopolysaccharide-protein antigenic complex has been isolated from a virulent, encapsulated strain of Past. multocida by extraction with cold, formalinized saline, and centrifugation at $105,000 \times g$. The original bacterial culture had been obtained from a bison that died of hemorrhagic septicemia (H.S.). Injection of fractional milligram amounts of the antigen into mice, rabbits, and calves produced toxic reactions that frequently killed the host. The surviving animals demonstrated a high degree of immunity to challenge with live, virulent organisms. Two injections with 15 μg of the antigen produced a high degree of immunity in

mice without the development of any signs of toxicity. The gross chemical composition and toxicity of the antigen were similar to those reported for endotoxins obtained by the Boivin of Westphal procedure. Although strong serological cross-reactions were obtained in Ouchterlony plates between the 105,000 x g antigens from the bison strain and an avian strain with antiserums to these strains, these antiserums agglutinated only the bacterial cells of the homologous strain. The active immunity obtained in mice by the injection of the 105,000 x g antigens of each strain was specific and could be correlated with the agglutination tests.

Pathogenic, immunologic, and serologic properties and biochemical reactions of 3 strains of Past. multocida from animals with hemorrhagic septicemia were studied (P-1256 Asia, P-1234 Africa, and M-1404 USA). Calves were infected when exposed by an aerosol, but the only calves that died were those in which septicemia was detected. Intramuscular exposure resulted in death in 24 to 48 hours. Pigs were highly susceptible to an aerosol and intranasal exposure of culture M-1404. Sheep were more refractory to culture M-1404 than were calves or pigs. Less than 10 CFU of each strain were lethal for mice. Calves vaccinated with strain P-1256 were immune when exposed to cultures P-1234 or M-1404. A calf vaccinated with strain P-1234 was immune when exposed to a culture M-1404. Calves that recovered after aerosol exposure were immune when re-exposed to heterologous cultures. Two strains of Past. multocida isolated from birds with fowl cholera, 1 from a calf with shipping fever, and 1 from a normal calf did not immunize calves against culture M-1404.

The 3 H.S. strains produced agglutinins in calves that were indistinguishable by the serum plate test with each of the 3 strains as antigens. Results of passive immunity tests, in mice, with absorbed and unabsorbed immune calf serum indicated that strain M-1404 was antigenically identical to strain P-1256, but not to strain P-1234. Slight differences in biochemical reactions were observed among the 3 strains. A culture, P-1459, isolated in September, 1965, from a young buffalo that died of H.S. at the National Bison Range, Moiese, Montana, was received after the above studies were completed. It was serologically similar to the Asian strain P-1256 and to the United States strain M-1404 that was isolated in 1922 from a buffalo in the Yellowstone National Park.

Gross and microscopic lesions observed in bovine and porcine H.S., and in bovine Past. multocida endotoxemia were determined. Widely distributed hemorrhages, edema, and general hyperemia were the most obvious tissue changes observed in the infected calves. Pneumonia was a constant lesion. Aerosol exposure produced a multiple focal fibrinosuppurative pneumonia, while intranasal and intramuscular inoculation resulted in generalized interstitial pneumonia. A slight lymphadenitis and degenerative changes in hepatic and renal parenchymal cells were also observed.

The predominant lesions observed in infected pigs were a diffuse, extensive, fibrinous pneumonia and fibrinous polyserositis. Edema and general

hyperemia were observed, but the widespread hemorrhages that occurred in the calves were not present. Acute lymphadenitis and renal tubule vacuolar degeneration were observed. A slight amount of cloudy swelling and focal areas of necrosis occurred in the liver.

Lesions in a calf that died following administration of Past. multocida endotoxin were widely distributed hemorrhages, edema, and general hyperemia. These lesions were especially evident in the lungs, and indicated widespread vascular alteration.

(Ames, Iowa) (ADP a7-25)

D. Newcastle Disease

At the Southeast Poultry Research Laboratory, Athens, Georgia, infectious bronchitis virus (IBV) interfered with the growth of Newcastle disease virus in cultures of chicken kidney cells. The destructive effects on the cells by Newcastle disease virus was prevented by an earlier inoculation of infectious bronchitis virus.

The interference of these two viruses was utilized in the development of a serological technique to identify the type classification of infectious bronchitis viruses.

Infectious bronchitis virus was more stable in certain salt solutions (MgSO_4 , Na_2HPO_4 , Na_2SO_4 , or K_2SO_4) but not in others (KCl , NaCl , CaCl_2 , MgCl_2) when subjected to temperatures of 50 C. for periods up to 80 minutes. This property of these salts seems to be related to the negatively charged ion (SO_4 or PO_4). It is hoped that further investigation will lead to the practical use of this phenomenon in stabilizing IBV biological preparations and procedures.

Preliminary research indicates that more realistic vaccine evaluation techniques may be necessary to determine the degree of protection offered broiler chickens by commonly used vaccination procedures.

Efforts are being directed toward the development of challenge and confinement techniques that facilitate the detection of subtle differences in disease susceptibility of chickens with divergent genetic backgrounds.

(Athens, Georgia) (ADP a5-35)

In cooperative research work at the University of Maine, the Specific Pathogen Free (SPF) Program for broilers and breeding hens, resulted in many advancements in the practical control of poultry diseases. A system of improved management practices, the use of killed Newcastle disease virus and other inactivated vaccines, together with accurate monitoring of diseases, through laboratory diagnosis have established that Newcastle disease and Mycoplasma gallinarum can be eradicated. Economic advantages, such as

lowering the condemnation rate, have been evidenced in the nearly 8,000,000 broilers under this study.

Infectious bronchitis has continued to be a problem, the solution of which holds priority in our future aims.

Marek's disease has become increasingly more evident and poses questions of origin and transmission that need intensive study.

(Orono, Maine) (ADP a5-28)

In cooperative research on Newcastle disease at the University of Wisconsin, a plaquing procedure has been developed for heterogenic strains of the virus.

In any quantitative study of the biology of a virus or the physical and chemical properties, the culture must be free of extraneous agents and consist of 1 genetic stock. The best procedure for obtaining a clone of virus has been the selection for propagation of material from a plaque produced by a virion growing in cells of a monolayer. Stocks of virulent strains of Newcastle disease virus (NDV) have been obtained in this fashion for some years. Most laboratory cultures and new isolates contain 2 to 4 populations that differ in their biological and physical properties. Unfortunately, lentogenic strains used in vaccines could not be examined in this way because they failed to produce plaques in monolayers of chick embryo fibroblasts. Addition of magnesium ions to the overlay provides an environment in which the lentogenic strains of NDV can produce plaques. Some of these strains were also heterogenous. It is now possible to plaque-purify vaccine strains and to establish the properties of the clones. By plating vaccine seed stocks under both magnesium-free and magnesium-containing overlays, it is possible to directly determine whether or not the seed is contaminated with virulent NDV.

A procedure of assessing virulence of NDV, the intracerebral inoculation of day-old chicks, has been further defined. Age in hours of the chick is important. Use of the yolk sac route rather than the allantoic sac route to inoculate 10-day-old embryos can appreciably speed up titration of lentogenic strains of NDV.

The repository for NDV continues to prepare supplies of fresh, lyophilized strains of the virus. Approximately 30 requests for virus or serum have been completed and mailed to the 30 requesting institutions.

(Madison, Wisconsin) (ADP a5-28)

Under a P.L. 480 Grant to the Veterinary Research Institute, Pulawy, Poland, research on Newcastle disease has produced the following results:

The changeability of biological properties of viruses and the relationship between hosts and viruses were studied in an attempt to determine the influence of various temperatures of hatching chick embryos on the changeability of pathogenic properties of the NDV.

The pathogenic strain of NDV (Radom) and the nonpathogenic strains (Roakin) were passaged 20 times. The results indicate that chick embryos incubated at 29-30 C. may constitute a convenient medium for the selection and replication of nonvirulent particles of the virus. This finding has been confirmed by pathological and histopathological examination of the dead embryos.

(Pulawy, Poland) (E21-ADP-13)

E. Susceptibility of Chickens to Respiratory Infections in Controlled Environments

Several types of environmental units have been developed by Agricultural Engineering Research Division personnel at the Southeast Poultry Research Laboratory, Athens, Georgia, for use in this project. Six cabinets with programmable air temperature, humidity, and separate wall temperatures have been constructed and put into operation. Initial trials in the absence of disease have compared the influence of wall temperatures on bird performance.

A prototype cabinet designed to conduct air transmission studies under a variety of environmental conditions has been constructed. A contract has been awarded for constructing 6 of these units.

Climatic controls have been provided for two of four 14 x 20 x 10' insulated, metal-clad rooms. A pressurized, filtered-air system has been added to several disease-free production houses. All intake air is drawn through high efficiency filters and maintained at 0.35 inch water column greater than outside to prevent infiltration.

An aerosol apparatus for exposure of birds to predictable concentrations of infectious material has been constructed and put into operation.

(Athens, Georgia) (ADP a5-37)

F. Relationship between Psittacosis Group Agents Found in Wild and Domestic Birds and Domestic Mammals

Circulating antibodies in the blood of turkeys, pigeons, sheep, rabbits, and a calf during the early and late stages of experimental infection with

bacteria of the genus Chlamydia (psittacosis group) were analyzed by sucrose-gradient centrifugation, electrophoretic, and heat methods. This analysis was made to determine whether the antibodies produced in the various animal hosts were similar. The sucrose-gradient centrifugation method separated the antibodies into 2 types, based on their characteristic sedimentation constant. These 2 types of antibodies reacted equally well in all of the serologic tests used to detect them. Electrophoretic analysis of the 2 types proved that both were of the gamma globulin variety of protein. But antibodies produced in calves were different from those produced in turkeys and sheep in their sensitivity to heating at 65 C. for 30 minutes. This finding indicated a fundamental difference in the chemical makeup of antibodies produced in these hosts.

(Ames, Iowa) (ADP a7-37)

G. Blue Comb Disease in Turkeys

In the cooperative work at the University of Minnesota, the study was concerned with the microbiological, physiological and related aspects of blue comb disease.

Intestinal material was collected from field outbreaks and laboratory cases of blue comb disease in turkeys. Such seed material was used fresh, and after frozen storage, to infect turkey poults and cell cultures of various types (avian, bovine, simian, human). An agent was isolated from pathogenic intestinal filtrates, which was cytopathogenic only to cells of turkey origin. However, no signs of blue comb disease were produced when such cell cultures were orally injected into turkey poults, nor was immunity to this disease induced in poults by such cell cultures.

Lyophilization as a means of preserving pathogenicity of filtrates and chromatographic column fractions was investigated and found satisfactory. The pathogenicity of bluecomb seed material must remain high for experimental work. Serial poult passage every 3 days maintained pathogenicity at nearly 100%.

Turkey poults under germ-free conditions show only reduced weight gains when given intestinal filtrates containing the blue comb agent. The agent does survive in germ-free poults, as filtrates made from infected germ intestines produce blue comb disease when injected back into conventionally-reared poults. Thus, intestinal bacteria seem to be part of the blue comb syndrome.

Bacteria of the genus Vibrio, isolated both in Canada and Minnesota from blue comb diseased birds, did not produce blue comb disease in experimental poults even with rapid serial passage and massive inoculums.

Mortality and morbidity from blue comb can be reduced by intentionally exposing turkeys through the drinking water to frozen, diluted, intestinal material gathered from mild cases of blue comb disease and the early treatment with milk replacer, muriate of potash, antibiotics, and adding heat until the birds are comfortable. Immunity to a second attack of blue comb disease is also conferred.

An attempt was made in 5 groups of turkeys to determine normal dry matter and water intake, excretion, fluid spaces of the body and changes occurring in several parameters during blue comb disease at the age of 8-9 weeks. These preliminary experiments indicate that blue comb disease in young turkeys is accompanied by the following: reduced or complete loss of dry matter intake, reduced water intake, weight loss, fall in rectal temperature, depression, reduced dry matter, and water excretion, increased percentage of water in excreta, increased or static packed red cell volume, no change in plasma water, reduced plasma Cl^- concentration, no change in T-1824 and NaSCN spaces, no change in the content of water in the whole body and no change in pH or pCO_2 of the blood.

These results indicate that dehydration from excessive loss of water and salts from the intestinal tract does not play a major role in the pathogenesis of blue comb disease in young turkeys.

Chromatographic columns using Sephadex, Sepharose, and ECTEOLA were used to fractionate intestinal filtrates made from the intestinal contents of turkeys sick with blue comb disease. The pathogenicity of the various column fractions was determined by injecting them orally into day-old turkey poults. Protein assays were also done on each fraction and total protein correlated with pathogenicity. A peak in the protein curve corresponded with pathogenicity. The pathogenic fraction came off the Sephadex and Sepharose columns immediately after the void volume.

Column fractions found pathogenic to turkey poults were also injected into various types of cell cultures including cells prepared from the whole intestine of turkey embryos.

Pathogenic intestinal filtrates were likewise injected directly into similar cell cultures. Although cytopathogenic agents were isolated in avian cell cultures from both column fractions and filtrates, blue comb disease could not be reproduced with either of such preparations nor could immunity to this disease be demonstrated when infected cell cultures were injected into day-old turkey poults.

(St. Paul, Minnesota) (ADP a5-31, (CA))

In cooperative research at the University of Wisconsin Agricultural Experiment Station, several virus isolations have been made from the intestinal material of turkeys showing signs of blue comb disease.

Attempts have been made to characterize and classify these isolates. The results of inoculation and immunoserologic studies suggest that this virus is involved with blue comb of turkeys.

(Madison, Wisconsin) (ADP a5-31, (CA))

Preliminary studies at the Texas Agricultural Experiment Station involved the development of techniques which may be used to qualitatively and quantitatively establish the gastrointestinal microflora of the "normal" turkey. Established "norms" are to be compared to the flora of turkeys clinically affected with blue comb.

(College Station, Texas) (ADP a5-31, (CA))

H. Paracolon (Arizona) Infections in Turkeys

In the cooperative research at the University of Minnesota, studies at 5 Arizona-infected premises indicated that the organisms will not survive for an extended period in the environment, provided Arizona-free turkeys are placed on the farm. For 2 years, Arizona-negative flocks have been maintained on previously infected premises.

Studies on known infected breeder flocks indicated that there is a rapid build-up of the infection during the latter half of the production period.

Vaccination experiments using 2 commercially available bacterins indicated limited value in protecting breeder flocks against egg transmission.

(St. Paul, Minnesota) (ADP a5-32, (CA))

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AREA NO. 6 - INFECTIOUS AND NONINFECTIOUS DISEASES OF FUR ANIMALS

Problem. In raising fur animals such as mink, rabbits, and foxes in captivity, disease problems incidental to the confinement of such animals are encountered. These include viral, bacterial, parasitic, mycotic, nutritional, and hereditary diseases. Virus diseases of mink cause the greatest loss to the 5000 mink ranchers now producing more than 7 million pelts annually valued in excess of \$130 million. The role of helminths as carriers of rickettsial and viral agents causing, or associated with diseases of fur animals, is becoming extremely important and one about which little is known.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving microbiologists and veterinarians engaged in both basic studies and the application of known principles of the solution of infectious and noninfectious diseases of fur animals. Research was conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3.4 scientist man-years. This effort was applied as follows:

Coordinated Field and Laboratory Studies on the Diseases of Fur Animals 1.2 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University, and under a cooperative agreement on encephalopathy of mink with the Wisconsin Agricultural Experiment Station, Madison.

Investigations on Diseases of Domestic Rabbits 0.2 under a cooperative agreement with the University of Arkansas, Fayetteville.

Transmission of Infectious Diseases by Helminths 2.0 at the Endoparasite Vector Pioneering Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 3.3 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Study of the Diseases of Fur Animals

Transplacental transmission of the Aleutian disease virus

Aleutian disease in mink is a transmissible viral disease which occurs in

families. In 17 bred females of the Aleutian genotype, known to have Aleutian disease, there were 29 dead and 30 live kits in utero. In 5 bred non-Aleutian females there were 5 dead and 23 live fetuses. Of the 53 live fetuses which were examined, 32 contained Aleutian disease virus. Transplacental transmission of Aleutian disease virus does occur.

Comparative studies on the Chediak-Higashi syndrome coagulation and fibrinolytic mechanisms of mink and cattle

The coagulation and fibrinolytic mechanisms were studied in mink and cattle with and without the Chediak-Higashi syndrome (C-HS). No defect was observed that would account for the hemorrhagic tendency in man, mink, and cattle with this syndrome. If the studies carried out on the mink and cattle also apply to man, the hemorrhagic tendency seen in the C-HS is not a direct manifestation of the gene or genes which control that syndrome, at least as far as the coagulation or fibrinolytic mechanisms are concerned.

Mink virus enteritis vaccines

Formalin-treated, inactivated cell culture vaccines (feline panleukopenia virus propagated in cell culture) were not antigenic. The test was performed in mink, using mink virus enteritis as challenge material. A similar test with an addition of an adjuvant to the vaccine again failed to protect the mink. The aerosolization of live, pathogenic feline panleukopenia virus as a vaccine failed to protect mink against mink virus enteritis challenge.

(Pullman, Washington) (ADP a6-7 (R))

Mink encephalopathy

Work to date on mink encephalopathy indicates that this disease is closely related to scrapie of sheep. If encephalopathy and scrapie are the same disease modified only by species variation, the mink probably become exposed by including infected sheep carcasses or by-products in their diet. Thus, the control of mink encephalopathy would be a necessary result of the scrapie eradication program. However, if the previous supposition is correct, a more inclusive question might be, "how has the recognition of mink encephalopathy broadened the epizootiological scope of the scrapie problem?" What other species might be affected? How might adaptation to 1 species alter the agent's communicability for others? Does the infective agent multiply in the reticuloendothelial system of only those hosts that it will ultimately destroy or is this characteristic independent of its lethal effect? Considering these questions with their wider implications and the extreme stability of the infective agent with which we are dealing, scrapie may have a, heretofore unrecognized, growing significance for our livestock industry.

(Madison, Wisconsin) (ADP a6-7 (R))

B. Abortion and Other Diseases of Rabbits

Epizootic abortion

Several areas of investigation have been started to determine the possible factors involved in the etiology of abortion in rabbits as it occurred during the period November, 1965, to June, 1966.

A preliminary study had been conducted in which 4 rabbits were inoculated with a mixture of bacteria isolated from a doe that had aborted 4 times consecutively. This inoculum included Mima polymorpha and a Gram-positive, aerobic bacillus. Three rabbits were inoculated with uterine washings of the same doe. Inoculation was done 25 days after breeding.

Four control does each kindled litters of 2-7 young each. One doe kindled 7 young from each of the challenge groups. One doe of the bacterial challenge group aborted 3 days later and M. polymorpha was isolated from the fetuses and placentas. One doe from each challenge group died with septicemia within 1 week of challenge inoculation.

These data suggested that abortion may be caused by an infectious agent but that the problem had not been duplicated experimentally. It seemed obvious that larger numbers of animals in each treatment group were required.

Thirty virgin does were obtained from a rabbitry with no previous history of abortion to be used in attempts to transmit the disease. The does were assigned equally to 3 treatment groups. However, the investigators were unable to produce abortion as it occurred in the field. All attempts to obtain additional field cases for isolation and identification and cross-infection studies have failed since no clear cut cases of abortion of the epizootic type have been reported within this period.

Additional attempts will be made to obtain potentially infectious material. However, certain conditions apparently were suitable for the induction of mass abortion in the winter of 1965-1966 that have not been present since that time.

Mucoid enteritis

Because mucoid enteritis constitutes an important economic loss to rabbit growers and as the specific nature and etiology of the disease is unknown, a detailed analysis of rabbits undergoing the disease process was begun. Initially, this analysis consisted of determining the total and differential leukocyte counts, body temperature, and packed cell volume of rabbits from the field. Early determinations of these parameters suggested that characteristic changes of an infectious nature did occur.

To gain additional information on the disease, rabbits were collected from a nearby rabbitry and from the University farm where rabbits were suspect. The total leukocyte count did not change consistently enough to provide a diagnostic tool. However, the lymphocytes were consistently reduced and the monocytes increased. These changes were most dramatic in rabbits that subsequently died, but were also observed in those that recovered and those that did not pass mucus. These data indicate that a characteristic change in mucoid enteritis involves cells of the lymphoid series and that littermates of rabbits grossly affected are also affected.

(Fayetteville, Arkansas) (ADP a6-9)

C. Persistence and Transmission of Viral and Rickettsial Diseases in Helminths

Adult intestinal flukes acquire rickettsial disease

The development of surgical techniques to "short circuit" the adult salmon poisoning disease fluke (Nanophyetus salmincola) cycle proves that rickettsia-free flukes can acquire the salmon poisoning rickettsia from infected dogs, and, further, can transmit this acquired infection to healthy dogs. This finding confirms the suspicion that an endoparasite can acquire an infectious disease from a definitive host.

(Pullman, Washington) (ADP P-1)

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AREA NO. 7 - MISCELLANEOUS INFECTIOUS AND NONINFECTIOUS
DISEASES OF ANIMALS

Problem. Included in this area of research are studies on problems involving more than one species of domestic animal, poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body; and bloat, a common, serious condition in cattle and sheep. Investigations of these diverse problems require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and noninfectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 28.2 scientist man-years. This effort is divided among subheadings as follows:

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Preparedness for Diagnosis of Foreign Animal Diseases 0.5 at the Plum Island Animal Disease Laboratory, Greenport, New York.

Biochemical Effects of Agricultural Chemicals 1.0 at the Toxicological Research Laboratory, Kerrville, Texas, and through a cooperative agreement with the Stephen F. Austin College at Nacogdoches, Texas.

Detoxication Mechanisms in Cattle and Sheep 1.0 at the Toxicological Research Laboratory, Kerrville, Texas.

Cytological Responses to Pesticides and Other Agricultural Chemicals 1.0 at the Toxicological Research Laboratory, Kerrville, Texas.

Toxicological and Pathological Effects of Pesticides 1.2 at the Toxicological Research Laboratory, Kerrville, Texas and through a cooperative agreement with the Texas Agricultural Experiment Station, College Station, Texas.

Mycotic Diseases of Domestic Animals 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chemical and Physical Studies on Microbial Antigens 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Microbiology of the Ruminant Digestive Tract and Its Relation to Digestive Disturbances 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Metabolic, Antigenic, and Pathogenic Characteristics of *Dermatophilus congolensis* 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Delineation of Motor Centers in the Brain that are Associated with Motility of the Ruminant Esophagus and Stomach 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Physiological Fate of Rumen Gases Absorbed from the Lungs Following Eructation 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Correlation of the Ultrastructural and Biological Properties of Animal Pathogens 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

The Effects of Mycotoxins on Animals 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Relationship between Psittacosis-Group Agents Found in Wild and Domestic Birds and Domestic Mammals 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Teratogenic and Toxic Compounds from Poison Plants 1.0 at the Poisonous Plant Research Laboratory, Logan, Utah.

Role of Parathyroid Hormone and Thyrocalcitonin in Calcium Metabolism 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Pituitary-Adrenal Function in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Toxicological Effects of Loco Plants on Livestock 1.0 at the Poisonous Plant Research Laboratory, Logan, Utah.

Development and Modification of Equipment for Greater Laboratory and Animal Room Safety 0.5 at the National Animal Disease Laboratory, Ames, Iowa.

Role of Physical, Chemical, and Biological Aerosols in Domestic Animal Diseases 0.5 at the National Animal Disease Laboratory, Ames, Iowa.

Teratological Effects of Carbamate Pesticides in Domestic Animals 1.0 at the Southwestern Veterinary Toxicology and Livestock Insects Research Laboratory, College Station, Texas.

Effect of Nitrates and Other Nitrogenous Compounds on the Toxicity of Pesticides to Livestock 1.0 at the Southwestern Veterinary Toxicology and Livestock Insects Research Laboratory, College Station, Texas.

Cellular Reaction to Intracellular Microbiological Agents 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Neurological Effects of Pesticides in Domestic Animals 1.0 at the Southwestern Veterinary Toxicology and Livestock Insects Research Laboratory, College Station, Texas.

Preparedness for Laboratory Assistance in Diagnosis of Duck Virus Enteritis (Duck Plague) 1.0 at the Plum Island Animal Disease Laboratory, Greenport, New York.

Toxicological Effects of Herbicidal-Treated Plants on Livestock 1.0 at the Poisonous Plant Research Laboratory, Logan, Utah.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 20.0 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Components of Normal and Immune Serums and Other Body Fluids

At the National Animal Disease Laboratory, serological and biophysical studies were conducted on fractions obtained by centrifugations of serums from several species of animals following exposure to chlamydial (psittacosis) agents.

The results of these cooperative studies are reported under Line Project ADP a7-37, Relationship Between Psittacosis-Group Agents Found in Wild and Domestic Birds and Domestic Animals.

(Ames, Iowa) (ADP a7-14 (R))

B. Preparedness for Diagnosis of Foreign Animal Diseases

The objective of this project is to develop and maintain adequate reference and diagnostic biologicals for use in diagnostic emergencies wherein

animals are affected with syndromes suggestive of infections not found in the United States. The following are examples of accessions examined during the year:

Contagious bovine pleuropneumonia was suspected in a dairy herd. The lesions seen at necropsy grossly resembled this exotic disease. The samples sent to the Plum Island Animal Disease Laboratory contained neither detectable Mycoplasmas nor antibodies to contagious bovine pleuropneumonia. A member of the genus Pasteurella, lethal for rabbits, was isolated from lung and thoracic fluid of 1 cow.

After 4 of 18 giraffes died at the quarantine station within 2 weeks, specimens from the giraffes were sent to PIADL. Specimens were tested for viruses, bacteria, Mycoplasma, and as a pool for infectivity for domestic ruminants. Other tissues were examined microscopically for pathological alterations, but findings were inconclusive. No virus or Mycoplasma was recovered. Bacteria were found and the only potential pathogens were identified as Salmonella.

(Greenport, New York) (ADP a7-16 (R))

C. Biochemical Effects of Agricultural Chemicals and Control Substances

Biochemical changes in plasma and tissue from exposure of animals to S-propyl-butylethylthiocarbamate as Tillam (R) and 2-ethyl hexyl ester of 2,4-dichlorophenoxyacetic acid as Weed Rhap (R), 2 herbicide compounds

Histopathological examinations of tissue specimens are still pending. Two sheep, dosed daily with 250 mg./kg. of Weed Rhap, showed decreases in plasma and tissue calcium:magnesium (Ca:Mg) ratio, which were not shown by the control sheep. This finding would indicate possible thyroid involvement.

Necropsy showed thyroid involvement in 1 sheep, but it was not apparent in the gross pathological observations during the necropsy of the 2nd sheep. Both sheep had kidney damage at necropsy.

Yearlings treated daily with 250 mg./kg. of Weed Rhap had no significant tissue ratio change. However, the yearling that died had a marked plasma ratio decrease while the 2nd yearling had a moderate ratio decrease.

Necropsy showed no gross pathological evidence of thyroid involvement. The kidneys were nephritic as was reflected by the 93% increase in the blood urea nitrogen (BUN) value.

The 2nd yearling was not killed. Therefore, necropsy findings are not available. This animal did have signs of poisoning consisting of partial anorexia, with a slightly increased BUN.

Two sheep treated with daily doses of 250 mg./kg. of Tillam had signs of poisoning. One sheep died from the treatments while the other recovered and was later killed.

The sheep had their greatest plasma Ca:Mg ratio change on the 4th day post-treatment, and this change was much greater than the change in the control sheep. The plasma ratios returned to pretreatment levels before termination of the test.

The sheep that was killed had a tissue ratio comparable to the ratio shown in the control sheep, and only a slightly increased BUN.

The gross pathological examination of this sheep showed only an enlarged liver and a distended gallbladder. No kidney or thyroid damage was noted.

The sheep that died because of the treatments with Tillam did have a decreased tissue ratio and a marked increase in BUN levels.

The gross pathological examination of this sheep showed no kidney or thyroid change.

Two Tillam-treated yearlings had a notable decrease in their plasma ratio levels. The tissue ratios of 1 decreased, whereas the 2nd yearling had an increase in magnesium in the tissue, but the calcium level failed to decrease. This animal, therefore, had an increased ratio.

Each of the treated yearlings had a marked increase in BUN. The gross pathological examination at necropsy showed chronic nephritis, an inflamed thyroid, congested lungs, and an enlarged liver.

It was not possible to necropsy the 2nd yearling.

Abate-treated cattle--Effect on enzymes

Cattle were given Abate, an organophosphorous insecticide (O,O,O',O'-tetramethyl-O-O'-thiodi-p-phenylene phosphorothioate), in their drinking water daily for 6 months. Two levels of Abate (10 and 20 p.p.m.) were utilized in the livestock drinking water. Three serum enzymes were studied to determine the effect of this compound, serum glutamic oxalacetic transaminase (SGO-T), aldolase (ALD), and creatine phosphokinase (CPK). No significant differences were detected in the activities of SGO-T and ALD in the groups. The CPK activity of the control group remained lower throughout the test than did either of the treatment groups. The group given water containing 10 p.p.m. daily had the intermediate CPK activity while the group given 20 p.p.m. had the highest overall activity.

Clinical and chemical tests of blood, urine and tissue specimens from pesticide-exposed animals

During the year, a total of 14,659 research specimens were tested by one or more clinical or chemical test procedures.

(Kerrville, Texas) (ADP a7-18 (R))

In cooperative research with the Physics Department, Stephen F. Austin College, work has continued on the development of a particle-size spectrometer for aerosols which depends upon the frequency dependence of the change in velocity of sound induced by the aerosol particles. Refinements have been made in the experimental techniques used for determining changes in the velocity of sound. These include: 1) Introduction of operational amplifiers to the electronic circuits to act as high- and low-pass filters to eliminate electrical noise; 2) Changes in the geometry of the tube in which the velocity of sound is measured to eliminate effects of reflections of the sound from the internal walls of the tube; and 3) the use of glass-wool sound sinks at the ends of the tube to reduce effects of reflection of sound both at the ends of the tubes and from objects elsewhere in the laboratory.

The present techniques should permit the collection of data with sufficient accuracy to ascertain the potentialities of developing a spectrometer based on the above described principle.

Work was done toward development of equipment to obtain microholograms of aerosols which may be used as an improved check on the operation of the particle-size spectrometer.

(Nacogdoches, Texas) (ADP a7-18 (R))

D. Detoxication Mechanisms in Cattle and Sheep

Survey to assure absence of water pollution

Several samples of well, creek, and river water from the area around the Kerrville Laboratory were collected, extracted, and checked by gas chromatography for the presence of chlorinated pesticides. No chlorinated pesticides were detected. Detection limits for 4 sample pesticides ranged from 0.25 to 2.5 parts per billion (p.p.b.).

Infrared spectrums of pesticide standards

A total of 171 infrared reference spectrums, including 69 pesticide standards, 17 solvents, and 85 other chemicals of various types, were run. The spectrums are to be used as reference material for study and interpretation.

Determination of simazine and hydroxy-simazine residues in tissue, blood, and urine of sheep

Simazine is a representative of the class of widely used chloro-substituted triazine derivative herbicides. Although these compounds are not used directly on livestock, there are possibilities that through grazing of sprayed vegetation, livestock could ingest the herbicides in sufficient quantity to provide eventual human consumption. Knowledge concerning absorption, elimination, metabolism, and residue deposition should, therefore, be established. In order to conduct such a study, it is first necessary to establish laboratory techniques to analyze the compound and its metabolite in the organs, tissues, and excreta concerned. The radio-metric technique was utilized solely for the probable metabolite, hydroxy-simazine.

(Kerrville, Texas) (ADP a7-19 (R))

E. Characterization of Cytological Responses to Toxic Actions of Pesticides and Other Agricultural Chemicals in Livestock and Poultry

Lesions resulting from repeated exposures to erbon [2-(2,4,5-trichlorophenoxy) ethyl-2,2-dichloropropionate] as Baron (R), a herbicide

The chlorophenoxy compound is of special interest as it has a greater toxicological effect than other compounds in this chemical group. Tissues from 4 sheep fatally affected by repeated exposures to a 41% erbon formulation were examined. The 4 sheep fatalities resulted following 6 doses at 50 and 100, 4 doses at 250, and 2 doses at 500 mg./kg.; all doses were based on technical equivalent and administered by oral capsule.

The histopathological changes were directly due to an agent toxic to the gastrointestinal mucosa, bringing about catarrhal enteritis. The extent of the disruption of cellular metabolism was sufficient to cause a loss of bacteriolytic function, permitting bacterial invasion.

(Kerrville, Texas) (ADP a7-20 (R))

F. Incidence and Pathology of Cancer and Other Tumors in Food-Producing Animals

The manuscript and 457 color illustrations for the Atlas of Meat Inspection Pathology have been submitted to the ARS Information Service for publication. This Atlas describes and illustrates 50 neoplastic diseases and 56 chronic infectious and noninfectious diseases, which resemble tumors, that are found in meat-producing animals.

consisting of dioxathion and dichlorovos and then given vitamin A, phenothiazine, and/or ascorbic acid. Although 2 cattle died, it was concluded that the factors investigated do not cause intoxication or adverse reaction when used with the insecticide spray.

Toxicological effects of plant insecticides to livestock

It was concluded that demeton is not a hazard to sheep when sprayed on crops or pastures at the recommended rate. Even though sheep may break into a field after the area has been sprayed, or the crop or pasture may be subject to spray drift, sheep detect the presence of the insecticide by 1 or more of the 5 senses and refuse to eat grass or forage that is thus contaminated. It is suspected that the commercial formulation of demeton is more readily detected by the sheep than is the technical compound.

Demeton offers an extreme hazard to cattle when sprayed on crops or pastures at recommended rates. Cattle do not differentiate between sprayed and unsprayed areas, will eat sprayed grass, and are poisoned.

From this year's work and from work in previous years, it may be stated that baby calves can withstand dermal applications of demeton at 0.01% and oral doses of 0.5 mg./kg. body weight without signs of toxicosis.

Carbophenothion in the feed of sheep causes a type of toxicity manifesting itself in muscular weakness, loss of control of the head, and a peculiar stance and head position when resting. These signs occur when the level of insecticide is at, or slightly below, 200 p.p.m.; however, when the level of the insecticide is increased to 400 p.p.m., the sheep refuse to eat the feed and will starve.

Mevinphos at 0.05% in baby calves is apparently a safe level for dermal application as is 0.1% in yearling cattle.

Toxicity of Organic Herbicides and Insecticides to Livestock and Poultry

Sixteen herbicide compounds have been screened, or are in varying stages of investigation, for their toxicity in cattle, sheep, and chickens. New data were collected on 46 insecticide compounds in cattle and sheep.

(Kerrville, Texas) (ADP a7-23 (R))

Cooperative studies were completed with the Texas Agricultural Experiment Station on tissue sections representing 142 experimental animals poisoned by 39 pesticide compounds. In general, the pathological effects tended to be characteristic of a chemically-related group of compounds rather than of individual compounds. The differences between the effects of compounds within a chemical family were quantitative rather than qualitative.

(College Station, Texas) (ADP a7-23 (R))

The primary purpose of the Atlas is to provide a readily available reference of the gross and microscopic features of 106 diseases that cause problems in the recognition of tumors found during meat inspection.

(Ames, Iowa) (ADP a7-22)

G. Toxicological and Pathological Effects of Insecticides, Herbicides, Fungicides, Larvicides and Other Agricultural Chemicals on Livestock and Poultry

Gas chromatographic determinations of Abate residues in water

The insecticide known as Abate (0,0,0,0'-tetramethyl-0,0'-thiodi-p-phenylene phosphorothioate) is a control measure for mosquito larvae in water. The only procedure available for the analysis of Abate was a colorimetric procedure. Therefore, efforts were made to develop a procedure utilizing the gas chromatograph for analysis. The water containing the insecticide was filtered through glass wool and extracted with successive portions of chloroform.

The chloroform extract was washed with alkaline solution to remove interfering materials. The extract was evaporated to a predetermined volume corresponding to a standard and was injected into the gas chromatograph. A hydrogen flame ionization detector was used in the analysis. The analytical column was a 5% Dow 11 on 60/80 chromosorb W in 1/8" x 6' column. Retention time was less than 3 minutes. Recovery was about 70% complete for the range of 0.5 to 20 p.p.m. The method detected concentrations as low as 0.05 p.p.m. in water.

Chronic toxicologic effects of Abate to cattle and sheep

Two cattle exposed for 12 months to a 420-mg. daily dose of Abate (1 to 1.5 mg./kg.) had mild signs of poisoning. Ten additional cattle were exposed to emulsion of 10 or 20 p.p.m. in their drinking water with no clinical effects. One heifer in each group produced calves that had abnormal development leading to early deaths.

Abate caused no ill effects in adult sheep or teratogenic effects on unborn lambs when administered to adults in gelatin capsules at 80 mg./ sheep for 422 daily doses, 5 mg./kg. body weight for 187 and 152 daily doses or 20 p.p.m. in drinking water for 184 and 176 consecutive days. Some cholinesterase depression occurred in some of the adult sheep, but was not accompanied by signs of poisoning.

Investigation of synergizing factors that may play a role in intoxication and adverse reactions to livestock following exposure to insecticide compounds

Thirty-four yearling cattle were sprayed with an insecticide formulation

H. Mycotic Diseases of Domestic Animals

Mycotic abortion is an infectious disease of cattle and horses. This disease has been recognized in many parts of the world and has assumed greater importance as other causes of abortion are controlled.

Investigations of the pathology and pathogenesis of this condition have been conducted with sheep as the experimental animals. Pregnant ewes were inoculated intravenously with a sublethal abortive dose of spores of Aspergillus fumigatus.

Placental tissues seemed highly susceptible to A. fumigatus infection as evidenced by the frequency of demonstrable infection (12 of 13 ewes inoculated), the morphology of the invading fungal elements, and the progressive nature of the placental lesions. In contrast, the sparsity of detected infection of maternal tissues, the retarded development of the infecting fungus, and the restricted nature of the maternal lesions suggested that maternal tissues were more resistant to A. fumigatus infection.

Lesions were found only in the skin and lungs in the fetus. This limited involvement of fetal organs and the histopathological findings suggested that infection of the fetus resulted from invasion by fungal elements in the amniotic fluid rather than by a hematogenous dissemination from the placenta.

When latex spheres were injected into the middle uterine artery of 2 ewes, 1 ewe had an abortion with infarction of the uterus and placentomata. The gross and microscopic lesions, produced by this mechanical interference with the blood supply, resembled those resulting from infection by A. fumigatus.

(Ames, Iowa) (ADP a7-24)

I. Chemical and Physical Studies on Microbial Antigens

A heat-stable, particulate, lipopolysaccharide-protein antigenic complex has been isolated from a virulent, encapsulated strain of Pasteurella multocida by extraction with cold, formalinized saline, and centrifugation at 105,000 X g. The original bacterial culture had been obtained from a bison that died of hemorrhagic septicemia, an infectious disease of cattle and buffalo. Injection of fractional milligram amounts of the antigen into mice, rabbits, and calves produced toxic reactions which frequently resulted in death of the host. The surviving animals had a high degree of immunity to challenge with live, virulent organisms. Two injections with 15 µg of the antigen produced a high degree of immunity in mice with no signs of toxicity. The gross chemical composition and toxicity of the antigen were similar to those reported for endotoxins obtained by the Boivin or Westphal procedure. Although strong serological cross-reactions were obtained in Ouchterlony plates between the 105,000 X g antigens from the bison strain and an avian strain with antiserums to these strains, these

J. Microbiology of the Ruminant Digestive Tract and Its Relation to Digestive Disturbances

A number of amino acids are deaminated and decarboxylated by ruminal micro-organisms and the acids produced are present in appreciable quantities in the rumen. Several species of important ruminal bacteria are able to reductively carboxylate and aminate these acids to resynthesize the amino acid from which the acid was originally produced. This new amino acid-organic acid cycle appears to have quantitative significance and evidence indicated more ruminal leucine was synthesized via carboxylation of isovalerate than by the biosynthetic pathway that has been demonstrated in aerobic bacteria. Earlier studies showed that these reductive carboxylations functioned in ruminal biosynthesis of leucine, valine, and phenylalanine. We have now found similar carboxylations operative in biosynthesis of isoleucine from 2-methylbutyrate and tryptophan from indoleacetate. Several species of anaerobic bacteria from environments other than the rumen have been examined for the operation of these biosynthetic reactions. Most of the organisms studied did not utilize these pathways but 2 species of photosynthetic anaerobic bacteria synthesized phenylalanine by carboxylating phenylacetate.

(Ames, Iowa) (ADP a7-30)

K. Metabolic, Antigenic, and Pathogenic Characteristics of *Dermatophilus congolensis*

Dermatophilus congolensis is the causative agent of an exudative dermatitis of several animal species including man. Electron microscopic studies were undertaken to obtain a more detailed understanding of the anatomical features of both motile and germinating cells of *D. congolensis* since they have been implicated in the spread and development of disease.

Intact and autolyzed motile-phase cells of *D. congolensis* were examined after negative staining with phosphotungstate. Motile-phase and cells in other developmental stages were examined in thin sections. The integument of motile-phase cells was homogenous and about 30 m μ thick and was associated with an outer diffuse capsule-like matrix. Three types of intracytoplasmic organelles and occasionally a large fluid-filled vesicle were present. Flagella varied from a few to more than 50 per cell; their diameter was estimated to be approximately 8-9 m μ . In autolyzed cells, the flagella were attached to differentiated regions of the cytoplasmic membrane. The peripheral zone of the integument of cells in all developmental stages appeared to undergo sloughing. In young hyphal and "packet" cells, stratification of the integument was frequently observed. Germinating cells of *D. congolensis* were larger than motile-phase cells and contained characteristic translucent membraneless cytoplasmic inclusions. The general features of *D. congolensis*, especially of germinating cells, closely resembled those of *Streptomyces violaceoruber*.

(Ames, Iowa) (ADP a7-32)

antisera agglutinated only the bacterial cells of the homologous strain. The active immunity obtained in mice by injecting the 105,000 X g antigens of each strain was specific and could be correlated with the agglutination tests.

The toxicity and development of resistance induced in mice by multiple intraperitoneal inoculations of the particulate antigens from each of 2 related strains of P. multocida (J. Bacteriol., Jan. 1967) have been determined and compared to those obtained with a Westphal preparation of Escherichia coli endotoxin. In each of the 3 homologous systems, 80-100% of the mice were protected against challenge inoculation of toxin which killed 20-100% of the mice in the heterologous systems and 80-100% of the controls. Inoculation of the mice with the P. multocida antigens produced a high degree of immunity only to challenge with live organisms of the homologous strain. The toxicity as well as the immunogenicity of the particulate antigens were lost by heating for 1 hour at 100 C. at pH 3, but no detectable changes were observed at 100 C. and pH 6. Since chemical analysis indicated the presence of glucose, galactose, heptose, and ester linkages in the P. multocida antigens, the immunizing component may be a toxic lipopolysaccharide which can induce a specific protective response to itself as well as to challenge with live organisms.

Since little is known about the development of active immunity by oral administration of killed microorganisms or extracts, a series of experiments was designed to determine the efficacy of whole cell antigen preparations of P. multocida given orally to chickens and turkeys. Formalin-killed saline suspensions of strain X-73 were administered in the drinking water or by pipette into the esophagus. In one of these tests, with 3 doses of antigen, 11 of 12 chicks were protected 10 days postvaccination against an intramuscular challenge inoculation which killed 14 of 15 controls. Relatively large doses of antigen were required to induce immunity by this method as compared to parenteral administration. Three doses were more effective than 1 or 2 doses containing the same total amount of antigen. Results of agar double diffusion tests and serum plate agglutination tests did not show any correlation with the active immunity obtained to the live organism. Serums from most of the birds that were immune were serologically negative.

These findings will contribute to the basic knowledge of the chemical and biological properties of the antigens of P. multocida.

This research has been carried out cooperatively with Line Project ADP a7-25, Investigation of the Genus Pasteurella.

(Ames, Iowa) (ADP a7-29)

L. Delineation of Motor Centers in the Brain That Are Associated with Motility of the Ruminant Esophagus and Stomach

Besides the work reported for the year ending June 30, 1966, some work has been done on recording methods. This research has been delayed because of the loss of our electronics specialist. We have a very recent replacement and so this work will be reactivated.

(Ames, Iowa) (ADP a7-33)

M. Physiological Fate of Rumen Gases Absorbed from the Lungs Following Eructation

The work on the absorption and fate of $C^{14}H_4$ was continued and proved to be very fruitful. Less than 1% of the radioactive methane was oxidized in the intact unanesthetized sheep. Radioactivity was measured in several carbohydrate moieties of the body.

(Ames, Iowa) (ADP a7-34)

N. Correlation of the Ultrastructural and Biological Properties of Animal Pathogens

A variety of structural alterations in Newcastle disease antigen are induced by physical and chemical agents. Antigen stored in phosphate buffered saline at 4 C. had 1) bleb formation and pleomorphic shape changes; 2) leaching of internal mass with increased permeability to salt solutions; 3) progressive denuding of surface antigen; and 4) rupture, often with the development of characteristic hemagglutinating particles. Antigen treated with "normal" chicken serum globulins induced pleomorphic shape changes. Ether treatment commonly caused an agglomeration of the surface antigen by stripping it away from the viral membrane. Antigen centrifuged into high salt concentrations and rapidly desalted was extensively altered in shape and frequently underwent disruption. Reaction of antigen with homologous antibody formed a close-packed, matted covering on the antigen. From the physical transformations observed, it was concluded that most antigen particles have a fluid-filled central region subject to osmotic deformation. The chemical inactivating agent, β -propiolactone, apparently renders the viral envelope more resistant to ether than the membrane of native virus particles. Reaction with partially purified antibody appears to enhance the antigen's stability by wrapping it in a tight proteinaceous meshwork.

"Whole" and autolyzed motile-phase cells of Dermatophilus congolensis were examined after staining with potassium phosphotungstate. In addition, both motile-phase cells and cells in other developmental stages were examined in thin sections. The integument of motile-phase cells was homogeneous and about 30 μ thick. It was generally associated with an outer diffuse capsule-like matrix. In the cytoplasm, 3 types of granules or

organelles were present. The flagellar diameter averaged 8-9 μ and the number of flagella per cell varied from a few to more than 50. In autolyzed cells, the flagella were attached to differentiated regions of the cytoplasmic membrane. "Sloughing" of the peripheral zone of the integument was common. In "young hyphal" and "packed cells," stratification of the integument was frequently observed. Germinating cells of D. congolensis were larger than motile-phase cells and contained characteristic translucent, membraneless cytoplasmic inclusions. It was concluded that the general anatomical features of D. congolensis, especially those of germinating cells, closely resembled Streptomyces violaceoruber.

(Ames, Iowa) (ADP a7-35)

O. Teratogenic and Toxic Compounds from Poisonous Plants

Four alkaloids have been isolated from the plant Veratrum californicum which are responsible for the cyclopien teratogenic effect in lambs born to ewes that ingested the plant during gestation. These 4 alkaloids were characterized by a variety of chemical techniques. Their structural similarities are currently under investigation. No other compounds of related chemical structure that were tested possessed this same activity. Various other alkaloids were also isolated from this plant and their teratogenic potential investigated.

Similarities between locoism and lathyrism were investigated. One of the known lathyrogens, aminoacetonitrile, produced the typical abortive and teratogenic effects common in animals ingesting the loco plant.

(Logan, Utah) (ADP a7-38)

P. Role of the Parathyroid Hormone and Thyrocalcitonin in Calcium Metabolism

Porcine thyrocalcitonin has been isolated and characterized. It was not similar to the previously reported porcine thyrocalcitonin. Material with thyrocalcitonin-like activity has been separated from normal porcine plasma. Further studies on the protective role of the thyroid in hypercalcemic states are in progress.

(Ames, Iowa) (ADP a7-39)

Q. Pituitary-Adrenal Function in Cattle

Adrenal venous blood was collected from unanesthetized calves. Adrenal corticosteroid secretory rates and adrenal blood flow rates were measured in unanesthetized undisturbed calves to give an estimate of basal adrenal function in these animals.

The effects of long-term infusions of Angiotensin on hemodynamic function and adrenal functions in calves have been examined. It was concluded that Angiotensin II per se is not solely responsible for the alterations in adrenal function which were observed previously in salt-depleted calves.

(Ames, Iowa) (ADP a7-40)

R. Toxicological Effects of Loco Plants on Livestock

One group of pregnant ewes was fed Astragalus pubentissimus from the 25th to 35th day of gestation and another group was fed this plant from the 25th to 50th day of gestation. To date, the time of insult appears to be chronologically nonspecific. Although skeletal malformations were produced, abortion was the principal effect of feeding loco plant to ewes. Blood alkaline phosphatase increased in loco poisoning.

(Logan, Utah) (ADP a7-41)

S. Development and Modification of Equipment for Greater Laboratory and Animal Room Safety

The value of expensive exhaust air filtration systems is immediately related to the "in use" monitoring programs designed to gauge the efficiency of the entire system under actual conditions of operation. Monitoring procedures employing viable biological agents, although efficient, have severe limitations as a result of the infectivity, decay rate, and residual contamination of the agent selected, as well as the time required to complete the assay procedures involved.

A simplified testing procedure employing small particle aerosols of a non-toxic, fluorescent dye, Rhodamine B was used to evaluate normal and physically-impaired filter mediums along with a biological procedure using T-3 coliphage. Results obtained in a test system, as well as those obtained in the evaluation of laboratory air filtration systems, indicated that the 2 procedures are comparable in efficiency and reproducibility. The fluorescent system was ultimately selected for all monitoring procedures used because of its greater speed and simplicity.

T. Role of Physical, Chemical, and Biological Aerosols in Domestic Animal Diseases

For many years, airborne transmission of hog cholera was considered of little significance. However, since the opening of the National Animal Disease Laboratory, a total of 16 different spontaneous outbreaks of hog cholera have occurred.

In the design of the large animal isolation facilities at the NADL, zones of differential air pressure were utilized to maintain isolation. No separation between the necropsy room and the corridor leading to the animal

isolation rooms was constructed. Isolation was to be maintained by a "static" air balance relationship between the 2 areas. Studies with T-3 coliphage conclusively demonstrated the impossibility of obtaining the desired control by this method.

The following factors also contributed to accidental aerosol transmission of hog cholera: 1) Deletion of automatic controls in the ventilation system; 2) loose-fitting ceiling-mounted light fixtures; 3) poor wall construction; 4) use of hollow clay tile; 5) unsealed openings in penthouse floor; 6) weakness in design concept; and 7) procedures that resulted in generation of high concentrations of airborne agents.

Physical alterations were made where possible and the operation of the buildings was modified to compensate for design deficiencies. No further outbreaks of hog cholera have occurred.

The influence of relative humidity (RH) on the survival of some airborne viruses was also studied. A modified toroid drum was developed for this work. Studies were conducted at 23 C. and at 3 RH levels (10, 35, and 90%) with 4 viruses (Newcastle disease virus (NDV), infectious bovine rhinotracheitis virus (IBR), vesicular stomatitis virus (VSV), and Escherichia coli B, T-3 bacteriophage (T-3 phage)).

Preliminary tests, utilizing Rhodamine B dye and NDV, were conducted to determine the physical, biological and total decay characteristics of the roller drum chamber. Aerosols were generated with a DeVilbiss 40 nebulizer and samples were collected with all-glass impingers. The collecting fluid was 1% peptone solution.

Newcastle disease virus was assayed in 10-day-old chicken embryos, IBR in primary bovine kidney cultures, VSV in primary swine kidney cultures, and T-3 phage by the plaque assay method in E. coli B cultures.

High humidity favored the survival of all 4 viruses during the aerosol generation process; however, considerable variation in survival during the storage period occurred. When stored at 23 C., NDV and VSV survived best at 10% RH. Infectious bovine rhinotracheitis virus and T-3 phage best survived storage at 23 C. at 90% RH.

(Ames, Iowa) (ADP a7-43)

U. Teratological Effects of Carbamate Pesticides in Domestic Animals

Daily doses of carbaryl (Sevin)^(R) were given to ewes at levels of 12.5 and 25 mg./kg. body weight for 1 to 6 weeks prior to conception and throughout gestation. There was no effect on reproduction and all lambs were born alive with no observable defects.

Blood cholinesterase studies conducted throughout this period indicated that each dose produced a transitory reduction of cholinesterase activity but there was no indication of a progressive or permanent lowering of the activity of the enzyme.

(College Station, Texas) (ADP a7-44)

V. Effect of Nitrates and Other Nitrogenous Compounds on the Toxicity of Pesticides to Livestock

Three experiments were conducted to determine the effects of ammonium salts during acute poisoning. Ammonium chloride was used in the 1st experiment, ammonium sulfate in the 2nd, and a mixture of ammonium chloride, ammonium sulfate, ammonium carbonate, and ammonium phosphate in the 3rd.

Signs of toxicity were muscular trembling at the onset of poisoning, which progressed to repeated strychnine-like tetanic convulsions in the advanced stages of poisoning. Death occurred during a final convulsion that appeared to result from respiratory failure. As the final convulsions developed, the body temperature increased sharply. Blood was found in the urine of several of the animals in advanced stages of poisoning.

Outstanding changes observed in the blood include a lowering of the pH of the blood and extremely elevated blood sugar level.

The outstanding gross pathological finding was severe active congestion of the lungs, severe hemorrhages of the thymus, numerous petechial hemorrhages of the heart, kidneys, and musculature of the body, and small hemorrhages in the spinal cord and brain.

In conclusion, the studies thus far have shown that excessive ammonium salts cause severe nervous signs, severe active congestion of the lungs, and extensive breakdown of the vascular beds in various parts of the body. These effects appear to be due to the ammonium portion of the salts independent of the anions. The significance of the elevated blood sugar and change in the pH of the blood to the signs and pathological findings is not known thus far.

(College Station, Texas) (ADP a7-45)

W. Cellular Reaction to Intracellular Microbiological Agents

Intracellular reaction, both in vivo and in cell cultures, has been studied using the following agents: contagious ecthyma, pseudocowpox virus, fowlpox virus, Gumboro disease virus, 2 viruses associated with porcine transmissible gastroenteritis (TGE), and the agent of psittacoid lamb polyarthrititis.

Findings have shown the primary cellular organelle for virus multiplication to be fat vacuoles (fowlpox), lysosomes (Gumboro disease), the cell membrane (TGE), and specialized viral-induced structures. The detailed

results and their possible significance have been integrated with previous knowledge concerning the pathogenesis of the respective diseases.

Studies on the mechanisms of viral release from cells shows that some are continually released (TGE in intestine) while others are not, but remain embedded and possibly protected in dead cell products (pseudocowpox embedded in keratin from the skin).

(Ames, Iowa) (ADP a7-46)

X. Neurological Effects of Pesticides in Domestic Animals

Weanling swine were fed carbaryl (Sevin)^(R), 1-naphthyl-n-methyl carbamate) in their diet daily for 12 weeks. All test animals were fed at a 150-mg./kg. level for 4 weeks and then the dose was doubled (300 mg./kg.) for half of the animals; the other half continued to receive 150 mg./kg.

When the dose was doubled after 4 weeks the animals had frank signs of intoxication after 9 and 16 daily doses at the higher level. The animals that received the lower level did not have signs of intoxication until 62 to 72 daily doses had been consumed.

Signs of intoxication were similar in all test animals and included a reluctance to stand, exaggerated lifting of hind legs when walking, staggering gait, prostration and inability to rise or stand, and finally death. Complete necropsies were done on each animal.

Microscopic examination of the tissues showed that there was cerebral edema, swelling of nerve cells, and progressive signs of nerve cell death in certain areas of the brain. In addition, several sections of muscle in each experimental animal showed that muscle fibers were undergoing degeneration and death. At necropsy, these muscles had the appearance of "white muscle"; that is, 1 muscle would be white while an adjacent muscle would have normal, red fleshed characteristics.

This "white muscle" aspect has been observed before in certain toxic conditions, but never in carbaryl-poisoned animals. None of the control animals had these changes.

Either or both of these degenerations -- brain or muscle -- could be responsible for the posterior paralysis seen in these animals. The significance of these findings at these high dosage levels is unknown but further studies are being prepared.

(College Station, Texas) (ADP a7-47)

Y. Preparedness for Laboratory Assistance in Diagnosis of Duck Virus Enteritis (Duck Plague)

Duck virus enteritis (duck plague) was isolated from ducks that died during an outbreak of the disease in January, 1967, on Long Island, New York. It was identified by using the serum neutralization test in duck and chicken embryos and it was differentiated from Newcastle disease and fowl plague. Examination by the serum neutralization test of domestic duck, wild duck, and geese serums received from all over the United States indicated that the disease incidence is limited to the duck farms in Suffolk County, New York. The disease was transmitted to susceptible ducks by intranasal, oral, and intracloacal inoculation. The virus was propagated in duck embryo cell culture with evidence of attenuation indicating the possible use of cell culture virus as a source for vaccine. The virus produced inclusion bodies in the liver and cloacal epithelium as well as in duck and chicken embryo cell culture. This criteria may be used as a method of diagnosis and identification of this disease. The virus was inactivated in 10 minutes' exposure at 56 and 60 C. Electron micrographs of the cell culture-propagated virus indicated that its size is 180 mμ, the dense inner core is about 75 mμ, and that it is a DNA virus. It may be classified as a member of the herpesvirus group.

Reference virulent and attenuated viruses and specific antiserum of high titer were prepared. In addition, illustrative material was prepared showing the disease signs and lesions which will be used as part of a film library for newly recognized and exotic diseases.

(Greenport, New York) (ADP a7-48)

Z. Toxicological Effects of Herbicidal-Treated Plants on Livestock

Feeding of Tordon-sprayed plants to calves and sheep

Many weed specialists have warned the public against the use of Tordon (4-amino-3,5,6 trichloropicolinic acid) because of its suspected toxicity to livestock. Recent research investigations have not found the chemical to be as toxic to animals as it is to plants. Tordon is a very effective soil sterilant and will kill many deep-rooted plants rendering the soil free from susceptible plants for 3 to 4 years following treatment.

Leafy spurge (Euphorbia esula) and alfalfa sprayed with 1 and 2 pounds of Tordon per acre did not cause any significant toxic effects on calves and sheep when ingested as the sole ration for 30 and 96 days.

(Logan, Utah) (ADP a7-49)

AA. Plants of the State of Sao Paulo Poisonous to Domestic Animals

A 5-year study under a P.L. 480 Grant to the Instituto Biologico, Sao Paulo, Brazil, was completed. The scientists developed information on a variety of poisonous plants indigenous to Brazil. They found one species of plant toxic for animals that they believed had not previously been so identified.

(Sao Paulo, Brazil) (S3-ADP-3)

BB. Effects of Prolonged Feeding of Terephthalic Acid to Rats

A 3-year study under a P.L. 480 Grant to the Hebrew University, Hadassah Medical School, Department of Experimental Medicine and Cancer Research, Jerusalem, is underway. A significant depression of growth occurred in both male and female rats when fed 5% terephthalic acid (TPA) for 50 days. Feeding the rats 2% TPA for 150 days caused a significant depression in the growth of male animals, and after 200 days' feeding of this percentage, both males and females showed highly significantly depressed weights. One percent TPA caused a significant depression in growth only in the female animals, the effect being evident after 150 days of feeding. In animals dying, kidney stones are usually observed.

(Jerusalem, Israel) (A10-ADP-8)

CC. Experimental Neurosurgical Problems

A 3-year study under a P.L. 480 Grant to the Veterinary Faculty, University of Ankara, Ankara, Turkey is underway on neurosurgical problems of domestic animals. The research studies under this project are of interest to scientists at the National Animal Disease Laboratory, Ames, Iowa, who are conducting studies on the biological changes associated with neuropathological conditions in livestock.

(Ankara, Turkey) (A22-ADP-9)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Toxicology

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AREA NO. 8 - FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth (FMD) and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of FMD was 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal diseases foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25% in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90% or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continue, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on FMD vaccines that are conducted cooperatively in The Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Agency for International Development, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The Federal scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 24.5 scientist man-years. This effort is divided among subheadings as follows:

Studies on Foot-and-Mouth Disease Virus 2.5.

Determine Mechanism of Antibody Formation 1.0.

Quantity Production of Foot-and-Mouth Disease Virus 2.0.

Establishment and Characterization of Cell Lines and Cell Strains 1.0.

Mechanism of the Interaction Between Foot-and-Mouth Disease Virus Molecules and Host Cells 2.0.

Genetic Biochemistry of Foot-and-Mouth Disease Virus 1.0.

Effects of Chemical and Physical Environment on Foot-and-Mouth Disease Virus 1.5.

Bulk Freeze Drying of Foot-and-Mouth Disease Virus Vaccine and Antiserum 1.0.

Identification, Purification, and Chemical and Physical Characterization of Foot-and-Mouth Disease Virus and Other Exotic Animal Viruses 2.0.

Immuno-Chemical Investigations of Foot-and-Mouth Disease Virus 1.5.

Attenuation of Representative Types of Foot-and-Mouth Disease Virus 1.5.

Biological Mechanism of Natural Resistance and Susceptibility to Foot-and-Mouth Disease Virus 1.5.

Biological Alteration of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures 1.0.

Morphological Aspects of Virus-Cell Relationships 1.0.

Diagnostic and Immunizing Procedures for Contagious Bovine Pleuropneumonia 3.0.

Carriers of Foot-and-Mouth Disease Virus 1.0.

Work was terminated under P.L. 480 Grant to the Instituto Biologica, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus (FMDV), and experimental field vaccination.

Under a P.L. 480 Grant to the Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey, research continues on studies of various indigenous types of FMDV, and the production of a vaccine for the control of foot-and-mouth disease in Turkey.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Interaction of FMDV Molecules and of Other Exotic Viruses with Host Cells

Inhibition of cell-free FMDV-RNA synthesis by antibody

Previous work had established the presence in FMDV-infected baby hamster kidney cells of a newly synthesized enzyme capable of functioning as a viral RNA polymerase in a cell-free assay system. It had also been reported that a 3rd antigenic component, distinct from the whole virus and viral protein subunit antigens, was demonstrable in infected culture cells and animal tissues. The present experiments establish that serums containing antibody to the new antigenic component inhibit the functioning of the viral RNA-polymerase in proportion to the amount of antibody. Polymerases from FMDV types A and O appeared to be immunologically related. The evidence indicates that FMDV-RNA polymerase may be the 3rd antigenic component.

FMDV-specific RNA replication

An FMDV-specific RNA polymerase has been solubilized with sodium desoxycholate. In the presence of dextran sulfate which inhibits RNase activity, the polymerase catalyzes the formation of 1) RNase-sensitive viral RNA, 2) RNase-resistant double-stranded RNA, and 3) a heterogeneous mixture of RNA precursors to viral RNA.

(Greenport, New York) (ADP a8-17)

B. Genetic Biochemistry of FMDV and Other Exotic Animal Viruses

Relationship between structure and biological function of FMDV

The amino acid composition of foot-and-mouth disease virus (FMDV), immunological types A₁₁₉, O₉, and C₃, was determined. The C-terminal sequence of

amino acids in the homogenous A₁₁₉ peptide was also investigated. High and low passage type A₁₁₉ viruses had slight differences in their content of 3 amino acids, in accordance with observed differences in their melting profiles, virulence, and electrophoretic mobilities. Virus types A₁₁₉, O₉, and C₃ differed significantly in their amino acid compositions but showed similarities as well. The basic, hydroxylated, and acidic amino acid groups comprised about 12, 17, and 19% of the protein, respectively. Half-cystine, present in the least amount (0.8%) of any amino acid, appears to exist in the protein coat in situ of type A₁₁₉ virus entirely as cysteine. Preliminary C-terminal sequence studies on type A₁₁₉ virus indicated a sequence of --serylalanylleucylglutamine.

Infectious nucleic acid from animal viruses

The infectivity of FMDV RNA was increased by several orders of magnitude by complexing the isolated RNA with the polycation DEAE-dextran prior to its assay in tissue cultures. Collaborative work was carried out which resulted in the isolation of infectious DNA from African swine fever virus and of infectious RNA from transmissible gastroenteritis virus of swine.

C. Identification, Purification and Chemical and Physical Characterization of FMDV and Other Exotic Animal Viruses

Production, concentration, and purification of FMDV

Production of purified FMDV, type A₁₁₉, from baby hamster kidney cells reached 0.1 gm. per week. Electrophoretic homogeneity was added to previous evidence for virus purity. The mobilities in veronal-acetate buffer at pH 8.6 and 0.1 ionic strength of low and high passage type A₁₁₉ virus — 3.28 and 2.84×10^{-5} cm²/volt·sec, respectively — were significantly different; but each increased on storage at 4 C. in a 0.05 M sodium phosphate at pH 7.5 to 3.75×10^{-5} .

Characterization of the polypeptides in FMDV protein

Certain evidence has been obtained by acrylamide gel electrophoresis for a single FMDV polypeptide. Aggregates of polypeptide of discrete sizes can be dispersed by treating the protein with 8 M urea, 0.1% sodium dodecyl-sulfate and 4 M ME. The ME not only serves to reduce cystine disulfide interactions, but also disperses regions of dense hydrogen bonding presumably due to the large content of threonine and serine present in the polypeptide chain.

Preliminary studies with CNBr fragmentation of FMDV protein yielded 5 to 7 components. Some of these are antigenic against rabbit anti-FMDV serum.

Electron microscopic chemical structure of African swine fever virus

Thin sections of pig kidney (PK₁₃) cell cultures infected with African swine fever virus (ASFV) were treated with enzymes in an attempt to identify the chemical composition of the morphological substructures of the virus. Ribonuclease and trypsin did not alter any component. However, the outer portion of ASFV including its hexagonal shell were removed by treatment with pepsin. Deoxyribonuclease removed the central dense core of the virus, confirming the finding that ASFV is a DNA virus.

Application of digital computers to ultracentrifugation

A computer program for processing moving boundary data from analytical ultracentrifuge analyses has been made generally available. The program has been updated to include the field-formed isodensity method. A computer program for finding the minimum of any function has been adapted for ultracentrifugation to investigate a) the Archibald method of molecular weight determination and b) the effect of concentration and pressure dependence on sedimentation rate.

(Greenport, New York) (ADP a8-25)

D. Immunochemical Investigations of Foot-and-Mouth Disease

The antigenic components occurring in FMDV infection were isolated and the serological reactivity established for each by complement fixation reaction. Antiserums specific for the individual antigenic components were prepared and evaluated by complement fixation and agar gel precipitin reaction. These specific antiserums provide valuable reagents for antigen measurement and antigenic analysis.

A procedure was developed for staining agar gel precipitin reactions with acridine orange. Used in conjunction with RNase and DNase treatment, the known single-stranded RNA nature of the nucleic acid of FMDV was readily confirmed. The virus protein subunit antigen and the infection associated antigen were shown not to contain detectable amounts of nucleic acid. The technique should be readily adaptable to other virus systems for purposes of identifying the precipitin band produced by the virus particles and for determining the nature of the nucleic acid in situations where this has not been previously established.

A new antigenic particle was described that is essentially the same size as virus particles but is devoid of RNA. The sedimentation coefficient (s-rate) and density of this new component were determined utilizing analytical ultracentrifugation procedures and the complement fixation test. The s-rate of this antigen was 75S and its density 1.31 g/ml. It appears to have unique antigenic properties in that it has both 140S and 12S antigenic reactivity.

(Greenport, New York) (ADP a8-26)

E. Biological Alterations of FMDV from Continual Residence in Cell Cultures

Three lines of investigation have been followed in attempts to obtain modified populations of FMDV which would not produce clinical disease in cattle but which could stimulate immunity.

Viruses kept in chronic residence in primary calf kidney cell cultures are FMDV, type O_{ML1} (33 months) and the Turkey variant of type A (16 months). In a recent test of the latter virus in cattle, only mild clinical signs were produced.

Serial passage of FMDV in dog kidney cell cultures resulted in rapid modification in the virus population characterized by a decrease in ability to infect cattle and development of capacity to interfere with propagating FMDV in calf kidney cell cultures. Residual noninterfering virus able to produce disease in cattle was separated from the total modified virus population by high-speed centrifugation. An immunizing strain that infects without producing lesions was developed from FMDV, type A₁₁₉, but modified immunizing strains developed from type A (Turkey variant) virus produced slight clinical signs of the disease.

FMDV, type A (Turkey variant) was adapted to growth in calf kidney cell cultures at low temperatures. Loss of infectiousness for cattle occurred after growth at 23 C., but residual virus with ability to produce disease in cattle could be separated from the total cold-adapted virus by high-speed centrifugation.

(Greenport, New York) (ADP a8-30)

F. Diagnostic and Immunizing Procedures for Contagious Bovine Pleuropneumonia

Preparation and use of a protein antigen in the indirect hemagglutination and complement fixation tests appear to give promise in increased specificity and application of these tests for the diagnosis of contagious bovine pleuropneumonia. Results of field studies showed the slide agglutination serum test in combination with the agar gel diffusion test was equally as sensitive and more simple to apply in the field than the complement fixation test. Vaccine studies have been initiated. Vaccinated animals were resistant to challenge at 1 and 2 months.

(Greenport, New York) (ADP a8-32)

G. Studies on FMDV (P.L. 480 Project)

Over a 5-year period, 492 samples from 349 outbreaks of FMD were tested. The work demonstrated the value of cell cultures, both primary and cell lines, in the laboratory diagnosis of virus from field samples. Passage of Type O virus, isolated from a naturally-infected pig, in a swine cell line, resulted in a loss of infectivity for cattle, but its immunogenicity also decreased in passage as indicated by the production of lower neutralizing antibody levels. Protection against a challenge inoculation, however, was not always related to the presence of a high neutralizing antibody level. The production of cell lines susceptible to FMDV was investigated. Cell lines of bovine kidney origin were less susceptible to FMDV than primary cultures of bovine kidney cells. Swine kidney cell lines were produced that maintained their susceptibility throughout the study. However, sublines of these cultures varied in their susceptibility. Genetic changes as shown by chromosome analysis (kariotype) accompanied the production of the cell lines, but no unique kariotype was associated with the degree of cell susceptibility.

(Sao Paulo, Brazil) (S3-ADP-2)

H. Studies on Indigenous Types of FMDV, and the Production of a Vaccine for the Control of FMD in Turkey (P.L. 480 Project)

Results have demonstrated once more that the classical challenge cannot be used as a normal challenge test on sheep as the control sheep do not regularly generalize their infection. The viremia test can be used as a challenge method but it is still an indirect method that needs much manipulation to be carried out. Index of protection, that is, the ratio between the virus infectivity titer on receptive sheep and the virus infectivity titer on vaccinated sheep, can be successfully used for challenge exposure of FMD vaccine on sheep.

Precipitating antibodies were interesting as long as they were present but their absence had no meaning.

(Etlik, Turkey) (A22-ADP-8)

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AREA NO. 9 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SWINE

Problem. Foreign diseases, such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is particularly important because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal, Spain, and France. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100%, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine for the purpose of developing information for increased protection of the Nation's swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3.0 scientist man-years. This effort is divided among subheadings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Greenport, New York.

African Swine Fever 2.0 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Kikuyu, Kenya, and in connection with a P.L. 480 Grant to the Patronata de Biologia Animal, Ministerio de Agricultura, Madrid, Spain.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Foot-and-Mouth Disease of Swine

Control of foot-and-mouth disease (FMD) in swine with commercial vaccines has not proved satisfactory. The use of an oil adjuvant with foot-and-mouth disease virus (FMDV) grown in tissue culture and chemically inactivated has afforded a degree of protection not previously available.

The response obtained with different virus types is not necessarily similar. However, it appears possible to combine a number of types to form a vaccine that will protect against the included virus types. Although current studies have not gone beyond protection for 90 days, it can be postulated that protection is afforded well beyond this point. With some FMDV types, vaccinated animals are more resistant to infection than animals recovered from the actual infection. A relatively simple, economical method of protecting swine from FMD is now available for consideration under field conditions.

(Greenport, New York) (ADP a9-1)

B. African Swine Fever

Studies were continued to explain the inability to detect serum neutralizing antibodies in pigs exposed to African swine fever virus (ASFV). Addition of rabbit antiporcine serum or complement to the serum neutralization test or treatment of ASFV with DNase did not demonstrate neutralizing antibodies. Electron micrographs of ASFV propagated in a cell culture in the presence of varying concentrations of hydroxyurea indicated that the central core of the virus particle was altered and appeared to be empty. The nucleic acid of ASFV was extracted and identified as DNA. Its resistance to thermal inactivation and the melting point behavior were characteristic of a double-stranded DNA. The DNA did not infect pigs but the cell culture-propagated progeny was lethal.

An additional method for the diagnosis of ASF was found. Leukocyte cultures prepared by known methods from the blood of infected pigs served as a sensitive and reliable indicator of the viremic state of the infected pigs, and was evidenced by the occurrence of hemadsorption and immunofluorescence in these cultures.

Although a completely safe and satisfactorily immunogenic African swine fever (ASF) vaccine has not been developed, fairly solid resistance can be produced in about 1/3 of the pigs. The development of chronic pericarditis and pneumonia prevents a larger percentage of pigs from being more satisfactorily protected. Resistance to infection could not be measured with the serological tests available.

(Greenport, New York) (ADP a9-2)

Under the terms of a P.L. 480 Grant, research is being continued at the Servicio de Patologia, Patronata de Biologia Animal, Ministerio de Agricultura, Embajadores, Madrid, Spain, on a rapid and accurate diagnostic method for ASF. The hemadsorption phenomenon may be closely associated with the intracellular virus replication.

The hemadsorption test for identifying ASFV (Malmquist and Hay test) largely used in Spain as a routine diagnostic method has given satisfactory practical results and has been a valuable aid in controlling this epizootic disease.

The 5 years of experience on the preparation of buffy coat cultures for the Malmquist and Hay test showed that this technique after it has been perfected and standardized in our laboratory, has given satisfactory results.

This technique is also a useful tool for the ASF studies, because it may be used safely and reliably in the large scale production of cultures.

Positive reactions specific for ASFV were obtained when suspected material from field studies was tested.

With this method it was possible to prove the presence of ASFV in 13,385 field specimens (spleen). Ninety-five per cent of the ASF cases were diagnosed within 1-4 days and 5% in 5-10 days.

Ninety-three per cent of the test-positive field specimens produced hemadsorption followed by cytolysis in the leukocyte cultures inoculated directly with this material. Seven per cent of the positive specimens produced only cytolysis without hemadsorption in these cultures but their hemadsorption capacity was recovered after 1, 2, or 3 subinoculations into fresh leukocyte cultures.

Usually the virus in the specimens of spleen taken from animals with the subacute or chronic form of ASF, or from carriers, did not produce hemadsorption in leukocyte cultures inoculated directly with this material but only the cytopathic effect. It was possible to identify this virus by serial subinoculations into fresh leukocyte cultures since hemadsorption generally occurred after 1 or 2 passages.

The virus in a few specimens (0.1%) caused cytolysis of the cultures, but the hemadsorption capacity was not recovered with serial passages. These strains usually recovered their hemadsorption capacity after 1 or more passages in swine.

The cytopathic effect without hemadsorption, produced by inoculating the spleen through more than 3 serial passages on leukocyte cultures, has always been produced by the ASF virus content of those inoculums.

The presence of the virus was confirmed by inoculating immune pigs against hog cholera.

In the Malmquist and Hay test, it is important to use nontoxic amounts of antibiotics for the buffy coat cultures.

The following end concentration in the culture may be tolerated: 500 I.U. of sodium G penicillin, 0.25 to 0.5 mg. of streptomycin and 100 to 200 U. of active Nystatin.

In the routine work, to obtain a final convenient concentration in the buffy coat cultures, the spleen suspension (inoculum) is treated with 4,000 U. of penicillin, 2.5 to 3 mg. of streptomycin and eventually 400 U. of Nystatin per ml. After 2 hours at room temperature, 0.2 ml. is inoculated into the culture tube with 2 ml. of fluid culture medium. The recommended concentration of the inoculum is 0.2 ml. This amount of antibiotics was enough to control the bacteria found most frequently in the field specimens.

(Madrid, Spain) (E25-ADP-4)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Foot-and-Mouth Disease of Swine

McKercher, P. D., Dellers, R. W., and Giordano, A. R. 1966. Foot-and-mouth disease infection in cattle housed in an isolation unit. Cornell Vet. 56:395-401.

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African Swine Fever

Addinger, H. K., Stone, S. S., Hess, W. R., and Bachrach, H. L. 1966. Extraction of infectious deoxyribonucleic acid from African swine fever virus. Virology 30:750-752.

Breese, S. S., Jr., and Hess, W. R. 1966. Electron microscopy of African swine fever virus hemadsorption. J. Bacteriol. 92:272-274.

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De Boer, C. J. 1967. Antibody studies in animals infected with African swine fever virus. Fed. Proc. 26: Abstract 1257.

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Coggins, L. 1966. Growth and some stability characteristics of African swine fever virus. Am. J. Vet. Res. 27:1351-1358.

Heuschele, W. P., and Coggins, L. 1965. Studies on the transmission of African swine fever virus by arthropods. Proc. U.S. Livestock San. A. 69:94-100.

Stone, S. S., and Hess, W. R. 1967. Antibody response of inactivated preparations of African swine fever virus in pigs. Am. J. Vet. Res. 28:475-481.

AREA NO. 10 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SHEEP

Problem. For the early detection of any outbreak of foot-and-mouth disease, comprehensive information regarding its effect on all susceptible species is necessary. The effect of foot-and-mouth disease (FMD) on cattle and swine has been, and is being investigated; however, little information is available pertaining to the disease in sheep. Sheep infected with FMD could serve as a source of infection and initiate the spread of the disease. Although primary research emphasis on exotic diseases of sheep at the Plum Island Animal Disease Laboratory is on FMD because of its great economic importance, other exotic diseases of sheep, such as rinderpest, sheep pox, louping ill, Nairobi sheep disease, and Rift Valley fever, are of concern to the Plum Island Laboratory because techniques and materials may be needed for diagnosis, control, and eradication on short notice and unexpectedly. Such diseases, if introduced into this country, could result in high death tolls or cause serious economic losses among susceptible sheep and other livestock. The problem is one of development of basic information applicable to protection of the nation's sheep from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases; and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department has recently activated a continuing and long-term program involving veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in some of the problems in this area.

The Federal scientific effort devoted to research in this area totals 1.0 scientist man-year. This effort is divided among subheadings as follows:

Foot-and-Mouth Disease of Sheep 1.0 at the Plum Island Animal Disease Laboratory, Greenport, New York.

Sheep Pox. Public Law 480 funds have been granted to the Turkish Ministry of Agriculture for a study of vaccines against sheep pox prepared from tissue culture propagated virus. The Madras Veterinary College, Madras, India, has also received P.L. 480 funds to conduct research on an efficient vaccine for protecting sheep against sheep pox. Sheep pox is indigenous in Turkey and India.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Preparation of a Vaccine Against Sheep Pox

Researchers in Turkey under P.L. 480 (A22-ADP-6) reported reasonable progress in the serial passage of sheep pox virus in cell cultures. Cytopathogenic changes are reported. A reaction at the injection site was observed in sheep inoculated with the attenuated virus. Animals subsequently challenged with a known pathogenic field strain of virus did not die.

(Ankara, Turkey) (A22-ADP-6)

In India, scientists under P.L. 480 (A7-ADP-5) have studied the immunogenicity of a sheep pox vaccine at several reactive doses. Nodular thickening and ulceration at the injection site have been observed. The keeping quality of lyophilized sheep pox vaccine kept at room temperature (26-30 C.) was tested at different intervals by titrating the vaccine in sheep. There was a titer reduction of 1 log after 45 days but no change in titer after 15 or 35 days. Injection of aluminum gel subcutaneously in sheep does not result in any undesirable reaction except in a persistent local nodular thickening.

(Madras, India) (A7-ADP-5)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

AREA NO. 11 - PARASITES AND PARASITIC DISEASES OF CATTLE

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 19.0 scientist man-years. This effort is divided among subheadings as follows:

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment 1.5 at the University of California, Davis, under a cooperative agreement with the ARS-USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan, Utah.

Host-Parasite Relationship of Intestinal Worms, *Cooperia* spp. in Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the Regional Animal Disease Laboratory, Logan, Utah.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Interrelationships of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with Emphasis on Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, University Park, New Mexico.

Effect of Stocking Rate and Rotational Grazing on Internal Parasitism of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Effect of Host Diet on the Bionomics of the Preparasitic Stages of Nematodes in Cattle Feces 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Life History and Host Parasite Relationship of Nematode Parasites 0.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Effects of Level, Rate, and Period of Exposure to Larvae on the Establishment and Pathogenesis of Gastrointestinal Nematode Parasites of Cattle 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Pathogenesis of Gastrointestinal Nematodiasis in Cattle 0.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Interrelationships of the Level of Parasitism and Stocking Rate of Beef Yearlings on Winter Temporary Pastures 0.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through cooperation with the Georgia Experiment Station, Experiment, Georgia.

Cytochemistry of the Enzymes of Eimeria stiedae 0.5 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Chromosome Studies of Various Species of Coccidia and Nematodes Parasitic in Ruminants 0.5 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Oxygen Uptake of Coccidia and Nematodes Parasitic in Ruminants and Laboratory Animals 0.5 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle, Sheep, and Alpacas) (P.L. 480 - Peru).

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle are under way through a P.L. 480 Grant at the School of Veterinary, Montevideo, Uruguay (P.L. 480 - Uruguay).

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 21.6 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment

At the School of Veterinary Medicine, University of California, Davis, under Cooperative Agreement 12-14-100-857(45) results were obtained as follows:

1. 1-Tetramisole used as a drench in calves at 8 and 12 mg./kg. was 94% to 100% effective in removal of Ostertagia ostertagi, Trichostrongylus axei, Cooperia spp., T. vitrinus, Nematodirus spp., and Dictyocaulus vivipara. There was no significant difference between the 2 dosage levels.
2. Co-Ral fed at 1.25 mg./kg. for 6 days in a molasses top dressing to range cattle was very efficient in preventing clinical gastrointestinal parasitism.
3. The susceptibility of sheep without a specific A-esterase in plasma to Haloxon toxicity has been reconfirmed.
4. The identity of the A-esterase with the so-called "Halon-high" factor of English workers has been confirmed.
5. The interference of Haloxon with wool growth in sheep negative for the plasma A-esterase following previous exposure to organophosphate compounds has been demonstrated.
6. The presence or absence of the A-esterase had no influence on the LD₅₀ of Co-Ral.

(Davis, California) (ADP 61-25)

B. Trichomonad Parasites

The Division's Regional Animal Disease Laboratory at Logan, Utah reported that antigenic studies reacting soluble antigens extracted from 6 strains of Trichomonas foetus with rabbit antisera against the 6 strains in agar gel by double diffusion revealed 12 to 15 precipitin lines formed in the micro method that was developed. This method is particularly suited to the reaction of small amounts of antigen and antibody.

(Logan, Utah) (ADP bl-26)

C. Host-Parasite Relationships of Intestinal Worms, Cooperia species in cattle

Workers at the Division's Regional Animal Disease Laboratory, Auburn, Alabama reported that a device was designed and constructed which permits relatively rapid separation of infective third-stage larvae from a large proportion of the debris which accumulates in Baermann funnels when fecal cultures are being processed. Larvae can be recycled through the apparatus as many times as necessary to achieve the required degree of cleanliness. This apparatus is efficient, easy to clean, and maintains larvae at a high level of viability.

(Auburn, Alabama) (ADP bl-27)

D. Etiology and Immune Response of Cattle to Winter Coccidiosis

At the Division's Regional Laboratory at Logan, Utah, scientists reported that the development of the first generation schizonts of Eimeria bovis was studied with electron microscopy in collaboration with H. G. Sheffield of NIH, Bethesda, Maryland, and with light microscopy. The details of formation of the merozoites from spherical bodies in the schizonts were observed. In collaboration with E. Scholtyseck of the University of Bonn, Germany, the fine structure of the macrogametes and microgametocytes of E. bovis and E. auburnensis was studied and compared with that of the similar stages of a pathogenic and a nonpathogenic species of rabbit coccidia. Some differences were noted among these species, but conclusions as to possible relationships between characteristics of fine structure and pathogenicity must await further study. E. bovis, E. ellipsoidalis, and E. auburnensis had different patterns of response with respect to the influence of temperature on excystation. Immature to mature schizonts of E. auburnensis were in epithelial cells of the crypts in the middle and upper small intestine in calves killed $6\frac{1}{2}$ to 12 days after inoculation. Growth of 1st generation schizonts to development of merozoites was observed in cultured monolayers of bovine kidney cells 14 to 18 days after inoculation of secondary cultures with sporozoites. Spleen and thymus cells also supported growth of the schizonts. Amprolium was effective in preventing coccidiosis experimentally caused by E. bovis in calves and by E. ninakohlyakimovae in lambs. (Logan, Utah) (ADP bl-29)

E. Bovine Anaplasmosis

Workers at the Beltsville Parasitological Laboratory report that in a 6-year period, bovine anaplasmosis was controlled and apparently eradicated from a beef herd in the semi-arid hill country of south-central Texas. Without resorting to the use of accelerated sale, slaughter, elaborate facilities, or a rigid tick control program, a heavily infected herd was replaced with a herd of anaplasmosis-free cattle that remained free of the disease for 2 years. Principal features of the program were the selection of noninfected replacement heifer calves from infected dams by complement fixation tests conducted 30 and 60 days after the calves were weaned and the separation in different pastures of CF-positive and CF-negative cattle. Sanitary veterinary procedures were followed to prevent accidental transmission of infection.

(Beltsville, Maryland) (ADP bl-30)

F. Parasites of Cattle, with Emphasis on Stephanofilarial Species

The Division's Regional Animal Disease Laboratory at University Park, New Mexico reported that stephanofilarial worms, which are transmitted by horn flies, cause unsightly sores on the ventral surfaces of cattle in many parts of the country. Almost 100% of mature cattle in many areas of the Southwest are affected. Preliminary tests showed that a Coral-Neguvon spray holds promise as a control measure for the parasite.

(University Park, New Mexico) (ADP bl-33)

G. Effect of Host Diet on the Bionomics of the Preparasitic Stages of Nematodes in Cattle Feces

Workers at Experiment, Georgia, under auspices of the Division's Regional Animal Disease Laboratory at Auburn, Alabama reported that the antioxidant used in commercial animal feeds (ethoxyquin, Santoquin, or EMQ) had an inhibitory effect on larval development in calf feces. Increasingly fewer larvae of the nematode Cooperia punctata were recovered from fecal cultures as the quantity of EMQ fed to infected calves was increased from the amount commonly used as a preservative ($\frac{1}{4}$ lb./ton) up to 25 times that amount. These results were comparable to those obtained in the case of C. pectinata, Trichostrongylus axei, T. colubriformis, Ostertagia ostertagi, and Oesophagostomum radiatum.

(Experiment, Georgia) (ADP bl-35)

H. Pathogenesis of Gastrointestinal Nematodiasis in Cattle

At the Beltsville Parasitological Laboratory it was reported that the optimum inoculum to establish infections in guinea pigs with the ruminant

nematode parasite, Trichostrongylus colubriformis, is 5000 larvae in a single dose. Repeated halving of the inoculum to as low as 625 larvae resulted in the establishment of the same average percentage of the dose. However, the most uniform infection within an experimental group was achieved with single doses of 5000 larvae.

Repeated doubling of the inoculum to 160,000 larvae resulted in lethal infections. As the number of larvae in the inoculum increased, the number of deaths within a group increased and the interval between inoculation and death decreased. A dose of 10,000 larvae was lethal in 11 days, of 20,000 in 10 to 11 days, 40,000 in 7 to 12 days, 80,000 in 4 to 7 days, and 160,000 in 48 hours to 6 days.

Oesophagostomum columbianum, a nematode parasite of the intestinal tract of sheep, developed to the 4th larval stage in cattle, and caused extensive lesions in the mucosa of the small and large intestines.

(Beltsville, Maryland) (ADP bl-38)

I. Environmental Factors Influencing Parasites and Parasitic Diseases of Economic Importance in Ruminants (Cattle, Sheep, Alpacas)

Under a P.L. 480 Grant, research was conducted at the School of Veterinary Medicine, University of San Marcos, Lima, Peru. Results were reported as follows:

1. Incidence of zooparasites of livestock in Peru. This work was done from 1962 to 1966 to gather information regarding parasites and parasitic species affecting domestic and wild animals serving as reservoirs of animal and human diseases.

A check list is presented, including taxonomy, geographical distribution by provinces and departments and the references of work done on the subject from 1885 to 1966.

This is the first complete report ever published about this matter and undoubtedly will be a good source of information for epizootiological studies in Peru.

2. Distribution of the most important parasitic diseases of livestock. Ten charts were prepared, showing the distribution of these diseases in the provinces and departments of Peru.
3. Climatic factors in relation to parasitism in the sierra, jungle and coast of Peru. Scientists were able to establish the relationship between climatic factors and the incidence and prevalence of parasitic disease of livestock in the coast, sierra and jungle of Peru. They also determined the strategic time for the application of preventive and control measures, in relation to the life cycles of the seasonal,

and incidence of external and internal parasites of livestock.

Charts and pamphlets were prepared and have been widely distributed among the farmers of these regions. They serve as the guide for parasitic control, in connection with the Ministry of Agriculture regarding diseases of livestock and the Ministry of Public Health, in relation to diseases transmissible from animals to humans.

(Lima, Peru) (S8-ADP-1)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle

Baker, N. F., and Douglas, J. R. 1966. Blood alterations in helminth infection. *Biology of Parasites*, E. J. L. Soulsby, Ed., Academic Press, N. Y. 155-183.

Russell, S. W., Baker, N. F., and Railzes, G. S. 1966. Experimental Obeliscoides curiculi infections in rabbits: Comparison with Trichostrongylus and Ostertagia infections in cattle and sheep. *Exptl. Parasitol.* 19:163-173.

Trichomonad Parasites

Johnson, A. Earl. 1967. Micro diffusion agar precipitin technique convenient for viewing and recording. *J. Bacteriol.* 93:1476-1477.

Host-Parasite Relationship of Intestinal Worms, Cooperia species in Cattle

Herlich, Harry. 1967. Effects of Cooperia pectinata on calves: Two levels of repeated oral inoculation. *Am. J. Vet. Res.* 28:71-77.

Etiology and Immune Response of Cattle to Winter Coccidiosis

Chobotar, Bill, and Hammond, Datus M. 1967. The asexual endogenous stages of Eimeria auburnensis. *J. Protozool.* 14 (Suppl.):21.

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_____, and Hammond, Datus M. 1967. Pinocytosis in the coccidium Eimeria auburnensis from cattle. J. Protozool. 14 (Suppl.):21-22.

Sheffield, Harley G., and Hammond, Datus M. 1966. Fine structure of first-generation merozoites of Eimeria bovis. J. Parasitol. 52:595-606.

Bovine Anaplasmosis

Roby, T. O. 1967. Anaplasmosis control in south-central Texas cattle. J.A.V.M.A. 150(12):1510-1512.

Parasites of Cattle, with Emphasis on Stephanofilarial Species

Hibler, C. P. 1966. Development of Stephanofilaria stilesi in the horn fly. J. Parasitol. 52:890-898.

Effect of Host Diet on the Bionomics of the Preparasitic Stages of Nematodes in Cattle Feces

Ciordia, Honorico, Porter, Dale A., and Bizzell, William E. 1966. Effect of ethoxyquin (Santoquin) on the development of the free-living stages of some nematode parasites of cattle. 41st Ann. Meet. Am. Soc. Parasitol., San Juan, P.R. p. 48.

Pathogenesis of Gastrointestinal Nematodiasis in Cattle

Herlich, Harry. 1966. Immunity to Trichostrongylus colubriformis in guinea pigs and lambs. J. Parasitol. 52:871-874.

_____. 1967. Effects of Cooperia pectinata on calves: Two levels of repeated oral inoculation. Am. J. Vet. Res. 28:71-77.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economic Importance in Ruminants (Cattle, Sheep, Alpacas)

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AREA NO. 12 - PARASITES AND PARASITIC DISEASES OF SWINE

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least \$200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 5.2 scientist man-years. This effort is divided among subheadings as follows:

Pathogenic Role of the Intestinal Roundworm 0.1 under a cooperative agreement with the Nebraska Agricultural Experiment Station, Lincoln.

Investigations of *Trichinella spiralis* 1.0 at the Beltsville Parasitological Laboratory and through a P.L. 480 Grant to the Polish Academy of Science, Wroclaw, Poland.

Strongyloides ransomi Infections in Baby Pigs 1.0 at the Swine Parasite Laboratory, Tifton, Georgia.

Biochemical and Other Aspects of the Host-Parasite Relationship in the Development and Severity of Helminthiasis in Swine 2.0 at the Beltsville Parasitological Laboratory.

Life Cycle of the Nodular Worm of Swine 0.5 at the Swine Parasite Laboratory, Tifton, Georgia.

Infection of the Dung Beetle, Phanaeus vindex, with Larvae of the Thick Stomach Worms of Swine 0.5 at the Swine Parasite Laboratory, Tifton, Georgia.

Swine Kidneyworms 0.1 under a cooperative agreement with the North Carolina Agricultural Experiment Station, Raleigh.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 4.6 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Intestinal Roundworms, Ascaris suum

The cooperative research at the University of Nebraska was concerned with the study of active and passive immunity to Ascaris suum in swine. No significant difference in the number of eggs of A. suum excreted in the feces was observed between the baby pigs from immunized and nonimmunized sows. Numerous eggs of A. suum were observed in the feces of the pigs from the 3 experimental groups.

A marked difference in average daily gain was not observed between the pigs in the orally-immunized group and pigs in the control group. However, the results suggest that the baby pigs from the antigen-immunized group made more significant average daily gains than did either the orally immunized or the control group.

The immunity resulting from experimental infections of X-irradiated larvae in swine was of no practical value, since all swine, including, challenged controls, developed severe clinical signs of ascariasis.

A marked elevation in the number of circulating eosinophils occurred in the pigs from groups 1 and 2 that received either 50,000 nonirradiated or X-irradiated infective eggs of A. suum. It reached the highest elevation 10 to 12 days after infection.

Twenty-two days after receiving 50,000 nonirradiated or X-irradiated infective eggs, the pigs in groups 1 and 2 were given 50,000 nonirradiated infective eggs. A very marked eosinophilia resulted which reached the highest elevation at 10 to 14 days.

Fewer and smaller larvae were recovered from the lungs of the pigs that received X-irradiated infective eggs. A significant difference in the number of larvae counted in the lungs of pigs immunized with X-irradiated or nonirradiated infective eggs of A. suum was not observed.

Double vaccination with various levels of X-irradiated larvae showed these results: Signs of ascariasis were not observed in 3 pigs that received 2 oral doses of 25,000 or 50,000 X-irradiated infective eggs of A. suum. The results suggest that a high degree of resistance to reinfection developed in the pigs immunized with 2 doses of X-irradiated infective eggs. This resistance was manifested at challenge by a marked difference in severity of clinical signs of ascariasis, and a marked difference in size of larvae in the lungs between immunized and control pigs.

(Lincoln, Nebraska) (ADP b2-12)

B. Trichinosis, *Trichinella spiralis*

Investigations were made on the pH of trichinous and nontrichinous fresh diaphragm and psoas muscle tissue from swine raised under controlled conditions. Infections of 25 to 350 trichinae per gram of diaphragm were obtained in the 5 experimentally infected pigs. A 6th was necropsied before the larvae could encyst and thus be counted. The pH of the trichinous pork ranged from 6.4 to 4.8 over 48 hours. The data indicated that the trichinous pork, held under the same conditions as the control samples, was generally more alkaline than the nontrichinous pork. This difference was more evident in swine 14, 22, 28, and 35 days after infection and was more marked in the diaphragm samples, than in those of the psoas muscle. The change in pH may be associated with the infectious process attending the invasion of the muscle tissue by the migrating larvae.

By holding trichinous pork at 0 F. for 20 days, before it was thawed and digested artificially, relatively clean cuticular material for use in the indirect fluorescent antibody test for trichinosis could be produced. After digestion, 98% of the larvae was completely digested and represented only by empty cuticle, whereas, the remaining 2% had ghost forms within the cuticle where the larvae had been situated. By repeatedly washing and sedimenting this residue, relatively clean concentrated cuticular material could be provided for this test. The availability of this material in quantity is important, as nematode cuticle is one of the principal binding sites used to detect the presence of antibody by the fluorescent antibody test.

(Beltsville, Maryland) (ADP b2-15)

Investigations on trichinellosis are also being continued under a P.L. 480 Grant to the Polish Academy of Science, Institute of Parasitology, Wroclaw, Poland, on the epizootiology, immunology, pathogenesis, and therapy of this disease.

Therapy in trichinellosis has improved in recent years. Symptomatic treatment consists of the use of Cortizole compounds or Azulen. The latter drug is effective without reducing host resistance. Romanian workers have reported an apparent beneficial effect of immune serums. Moreover, at least 2 compounds, Thiabendazole (Merck, Sharp and Dohme) and Neguvon (Bayer), exert a strong parasitocidal effect on migrating and encysted larvae. Few therapeutic trials with Thiabendazole have been made in human beings.

In a search for a simple diagnostic test suitable for field conditions, researchers have tried the charcoal card test. The kit of reagents was supplied by a firm in the United States. In comparative studies performed with 179 positive or suspect serums and 65 negative serums, the charcoal card test was less sensitive than other serological tests. However, it is very easy to perform.

In recent years, fluorescent antibody tests have increased in importance. A modification of the indirect method was developed using larval cuticles counterstained with gentian violet as antigen. As a slide method, the test is simpler than the previous tube procedure with the added advantage of reagent economy. It has higher sensitivity and specificity, if requirements are met.

In spite of numerous serologic studies, the persistence of antibodies after T. spiralis infection has not been assessed. Therefore, 12 rabbits infected with Trichinella were studied for 2 years and 9 months. Six different tests were used to follow the time of antibody appearance, increase in titer, and its loss. At the last examination, all rabbits had a positive reaction. Similar studies were made in 2 groups of human beings (23) with trichinellosis for 254 days after infection. Positive serologic results were detected by sensitive tests in 19 persons infected 7 years ago, in 21 persons infected more than 9 years ago, and in 19 persons infected 19 years ago. All tests pointed to a prolonged presence in immune antibodies.

(Wroclaw, Poland) (E21-ADP-9)

C. Strongyloides ransomi Infection in Baby Pigs

Two identical experiments were performed in the spring and fall of 1966 to compare S. ransomi infection in Duroc, Hampshire and crossbred pigs.

In each experiment, 6 pigs of each of the 3 breeds were exposed to 3 million infective larvae of S. ransomi and 6 similar pigs were maintained as controls.

A difference was noted in the average rate of gain and feed conversion factor among breeds. The crossbred pigs had a higher average daily gain (1.58) and were more efficient in converting feed into weight gain (3.40) than were the other 2 breeds. The Hampshire pigs were the poorest gainers (1.35) and the least efficient in feed conversion (3.58). The Duroc pigs were intermediate in gain (1.50) and efficiency (3.47).

The greatest effect of parasite infection on rate of gain was noted in the Hampshire pigs with a difference of 0.46 in favor of the control pigs. The least difference was noted in Duroc pigs (0.20). The crossbred pigs were intermediate in difference in rates of gain (0.31).

(Tifton, Georgia) (ADP b2-17)

D. Infection of the Dung Beetle, *Phanaeus vindex*, with the Larvae of the Thick Stomach Worms of Swine

Beetles were collected in 3 different ecological areas by means of baited coffee cans buried in the ground. The areas trapped were: swine pastures, dairy cattle pastures, and a swamp and woods area. Feces of 8 different animals were used as bait in each location to assess possible beetle food preference. Fresh feces in equal amounts were used from swine, opossum, dog, raccoon, cattle, horse, chicken, and lamb.

Results showed that dung beetles, and particularly *Phanaeus vindex*, the most common intermediate host for thick stomach worms of swine, are more strongly attracted to feces of omnivorous animals than to feces of herbivorous animals. More dung beetles were attracted to swine or opossum feces than to that of other species, even in an environment dominated by cattle. The latter findings were totally unexpected since most citations in the literature on dung beetles state that their environment is in cattle droppings.

(Tifton, Georgia) (ADP b2-20)

E. Swine Kidneyworm, *Stephanurus dentatus*

Prenatal infection of pigs by the swine kidney worm, *Stephanurus dentatus*, has been demonstrated in 4 trials in which infective larvae were given to gilts during pregnancy.

Lesions produced by swine kidney worms and *Ascaris suum* can be differentiated by gross and microscopic examination. Diagnosis of infection by *S. dentatus* should prompt the owner to instigate a program using gilts for 1 farrowing and retaining no older swine on the premises to spread the infection.

A circular was prepared in cooperation with the Extension Service describing the gilt-only method. This circular was widely distributed through the state.

(Raleigh, North Carolina) (ADP b2-21)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Intestinal Roundworms

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Trichinellosis

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Infection of the Dung Beetle

Stewart, T. Bonner, and Davis, Robert 1967. Notes on mites associated with coprophagous beetles. J. Ga. Entomol. Soc. 2:21-26.

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Batte, E. G., Moncol, D. J., and Barber, C. W. 1966. Prenatal infection with the swine kidney worm (Stephanurus dentatus) and associated lesions. J.A.V.M.A. 149:758.

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AREA NO. 13 - PARASITES AND PARASITIC DISEASES OF SHEEP AND GOATS

Problem. The cost of parasitic diseases to the sheep and goat industry of the United States is estimated to be in excess of \$45 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult, and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy animals, insure adequate supplies of high quality lamb for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, parasitologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of parasites and parasitic diseases of sheep and goats. Research is being conducted on these diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 6.2 scientist man-years. This effort is divided among subheadings as follows:

Gastrointestinal Nematodes 1.1 at the Beltsville Parasitological Laboratory, and under a cooperative agreement with the Kentucky Agricultural Experiment Station, Lexington.

Life Cycles of Sheep Coccidial Parasites 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Effect of Intestinal Roundworms on the Tensile Strength and Sulfur Content of Wool 0.1 under a cooperative agreement with the North Dakota Agricultural Experiment Station, Fargo.

Chemical Control of Sheep Nose Bot, Oestrus ovis 1.0 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Biology and Control of Psorergates ovis, the Australian Itch Mite of Sheep 0.5 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Pathobiology of Laboratory and Field Strains of Psoroptes ovis 0.5 at the Parasite Research Laboratory, Albuquerque, New Mexico.

The Biology and Control of Liver Flukes 1.0 at the Parasite Research Laboratory, Las Cruces, New Mexico.

Biology and Control of Helminth Parasites of Sheep in the Southwest 1.0 at the Las Cruces, New Mexico, field station, and through informal cooperation with the New Mexico Agricultural Experiment Station.

Immunological Response of Sheep and Goats to Strongyloidiasis, a P.L. 480 Grant with the Zoological Institute, University of Warsaw, Warsaw, Poland.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 11.9 scientist man-years.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

A. Gastrointestinal Nematodes and Nematodiasis of Sheep

The results of 3 experiments using 49 lambs indicated that colostrum-deprived, parasite-free lambs were more susceptible to gastrointestinal parasitism than lambs raised to weaning age with their dams. In 1 experiment, all lambs were exposed to natural infection with miscellaneous internal parasites while grazing a pasture contaminated with infective larvae. In the other experiments, the lambs were artificially infected. In one, they were reinfected with comparable numbers of the larvae of the large stomach worm, Haemonchus contortus. In all instances, the colostrum-deprived, parasite-free lambs either had a lower rate of gain or had lower hematocrit levels than the lambs raised with their dams. The worm loads of the parasite-free lambs were similar to or less than those of the lambs raised with the ewes.

The results of 2 experiments in which 7 pairs of lambs were used, indicated that lambs given iron-dextran before and after experimental infection with the large stomach worm, Haemonchus contortus, usually harbored fewer worms at necropsy than comparable nontreated lambs. Six of the 7 lambs given iron injections had fewer worms than those not given the injections. There were no significant differences in weight gains or hematocrit levels.

The 4 species of nematodes in lambs from the Meat Animal Research Center, Clay Center, Nebraska, are reported for the 1st time from sheep in that state. They are Nematodirus abnormalis, N. lanceolatus, N. helvetianus, and Trichuris discolor. This report also constitutes the first finding in sheep of T. discolor, which is a parasite normally occurring in cattle.

(Beltsville, Maryland) (ADP b3-16 (R))

In cooperative research at the Kentucky Agricultural Experiment Station the organic phosphate compound, trichlorfon, given subcutaneously, was generally less effective for removing gastrointestinal nematodes from lambs than the standard anthelmintic, thiabendazole, used as a reference. Although trichlorfon was more active against whipworms and compared favorably with thiabendazole for mature common stomach worms and small intestinal cooperids, it was notably less effective against other species of stomach and intestinal nematodes and the immature stages of all of the species encountered.

The ability of dietary protein supplementation given to calves to counterbalance their increased exposure to mixtures of gastrointestinal nematodes was evaluated. Supplementation with 16% crude protein-pelleted feed given at the rate of 1 kg./calf/day prevented clinical signs of parasitism at the exposure level used. However, the supplementation did not completely counterbalance the influence of the helminth infection on the blood serum protein constituents. These changes in serum proteins were quantitatively in the same direction seen in animals known to have succumbed to parasitism and were interpreted as a subclinical reaction of the calves to injury or stress caused either directly or indirectly by the helminth parasites.

(Lexington, Kentucky) (ADP b3-16 (R))

B. Life Cycles of Eimeria ahsata and E. intricata (Sheep Coccidia)

Each of 2 lambs was given 80,000 oocysts of Eimeria ahsata and was killed on the 9th or 14th postinoculation day to obtain additional data on the life cycle as revealed by electron microscopy. Two others were given 50,000 oocysts of E. intricata and were killed on the 10th and 14th day respectively. Glutaraldehyde, 6%, was chilled in ice and injected immediately after death into the ileum, tied off in 2 places, about 1 foot above the ileocecal valve. The 3"-length of intestine was distended by injecting sufficient amounts of cold glutaraldehyde to fill the lumen, thus stretching the tissue and fixing rapidly at the same time. The 3"-length of ileum was then excised and placed in 3% glutaraldehyde, was packed in crushed ice immediately, and was then opened while subsequently keeping the tissue and solutions chilled in ice or in a refrigerator.

Half of the tissue samples were transferred as soon as possible into a small quantity of 3% osmium tetroxide and, by using blunt dissection, the amount of tissue to be preserved was reduced by scraping off the inner surface of the intestine and discarding the middle and outer layers. The mucosal layer was sliced into small bits while still in cold osmium and transferred to tiny vials for transferring through dehydrants into either Maraglas or Vestopal embedding mediums for electron microscopy.

The remaining half of the tissues were handled as above, but the slicing and preserving were done in cold 3% glutaraldehyde and the bits of tissue

were retained in the same solution in vials in the refrigerator overnight before post-fixing in osmium. These were then handled as before, finally embedding in either Maraglas or Vestopal. Fresh, unfixed tissues were preserved in liquid nitrogen at -160 C. and were freeze-dried for indefinite storage at -190 C. for future studies with the electron microscope. These sections and others will be examined later by electron microscope specialists.

(Auburn, Alabama) (ADP b3-19)

C. Effects of Intestinal Roundworms on Wool Quality

Sixteen lambs with moderately high numbers of intestinal nematodes, primarily Trichostrongylus sp., were divided into 4 lots as equally as could be achieved. Two lots were treated with thiabendazole to eliminate the worms. The study was conducted for 7 weeks following medication. Medicated lambs had no nematode ova in the feces and the others averaged 2433 ova/g. of feces. There were no statistical differences among groups in weight gains or the pH, glucose, pyruvate, β -hydroxybutyrate or acetone of the blood.

(Fargo, North Dakota) (ADP b3-20)

D. Chemical Control of Oestrus Ovis

Attempts to raise Oestrus ovis larvae in vitro are progressing slowly. One of the main difficulties is the apparent lack of hardiness of the 1st instar. In nature, under the most favorable conditions, after having spanned the hazardous gap between the abdomen of the gravid female fly and the nasal passages of a suitable host, there is still only 1 chance in 10 that the larva will survive the first ecdysis. Even those larvae that do not actually succumb may remain dormant for as much as 11 months after attempting the maturation process. When one is trying to maintain these frail creatures in a foreign, and perhaps hostile, environment, the chances of success are even more problematical. It is not known, for instance, if larvae will survive the rigors of collection from slaughtered sheep at an abattoir.

Nutritive mediums designed to support larvae become an ideal environment for the multiplication of bacterial and fungal contaminants. These organisms act directly on the larvae as pathogens, or render the medium unsuitable, causing the larvae to die. Since strict asepsis is observed in all steps of medium preparation, it is obvious that the contaminants are introduced with the larvae. One of the problems, then, is to find some way to decontaminate the larvae before they are placed in the medium.

(Albuquerque, New Mexico) (ADP b3-23)

E. Biology and Control of *Psorergates ovis*, the Australian Itch Mite of Sheep

On July 1, 1966, the *Psorergates ovis*-infested flock at the Parasite Research Laboratory, Albuquerque, N. M., consisted of 44 sheep (25 ewes and 19 spring lambs). Eight ewes were then infested with *Ps. ovis*.

In September, reexamination of the flock revealed that 2 lambs were not infested. Three of the 8 infested ewes were then examined, but no live mites were isolated.

The flock was left undisturbed until June, 1967, by which time 2 of the 8 ewes previously infested, and 2 noninfested ewes, had died. By an exacting standardized procedure, skin scrapings were collected from each of the surviving 21 ewes and 19 yearlings. A total of 280 microslides were prepared from 120 skin scrapings, representing widely distributed skin surfaces. Despite this painstaking effort, live mites were isolated from only 2 sheep. Both sheep were among the 8 ewes infested a year earlier.

Strangely enough, among negative sheep (pending further investigation), was an aged crossbred Rambouillet range ewe that had been the parent host of the present *Ps. ovis*-infested flock and was infested continuously from 1963 through 1966.

In the spring of 1967, 17 lambs (predominantly of Rambouillet-Suffolk breeding) were born in this flock but were considered too young to be examined in June. These lambs will be examined for infestation for the first time in July, 1967 (at an average age of 4 months).

Under the circumstances, it appears unlikely that a high incidence of *Ps. ovis* can be expected in this experimental flock in the near future. Because this is the only known infestation in the USA, experimental subjects will not be used for related studies until the parasitism has attained more reassuring proportions.

(Albuquerque, New Mexico) (ADP b3-24)

F. Pathobiology of Several Laboratory and Field Stains of *Psoroptes ovis*
The Mite of Common Sheep Scab

Population components of *P. ovis*

The phenomenon of summer latency, or dormancy, of *Psoroptes ovis* infestations on sheep (and on cattle as well) has long been recognized. Population pressures of scabies mites subside each summer, and may or may not rise again in the fall. The summer factors that are responsible for the apparent recovery of the host have not been established. Studies on the periodic latency of scabies have, during recent years, resulted in interesting

contributions. The present study, reported here, is designed to determine which components of the mite population infesting sheep can oversummer and perpetuate the species.

As of June 30, 1967, this study has progressed 20 months. During the present fiscal year, workers could not collect as many mites monthly as during the first 8 months of the investigation (206 mites/month as against 583 mites/month; the monthly average collection for the 20 months is 357 mites).

It is anticipated that this program will continue through November, 1967, completing a 2-year period. An interpretation of our data reveals that the larval stage is the most populous and it reached a peak in July of 1966. In May and June, 1967, it again increased and will probably peak in July, 1967, as in the previous year. From these observations, it appears that the ovigerous female mites deposit more eggs in the late spring months than at any other time of year; therefore, an abundance of larvae is available for the difficult period of summer survival.

Another noticeable feature is that each ovigerous female stage peak (except one), was preceded by an adult male peak. And perhaps the last significant characteristic would be the prolonged, sustained, high population of the ovigerous female stage immediately before the perilous summer season occurs.

Candidate acaricides against P. ovis

In the 1966 fiscal year, several candidate acaricides were tested for their effectiveness in eradicating infestations of the common sheep scabies mite, P. ovis.

Results of completed tests strongly suggest that Prolate (Imidan, O, O-dimethyl S-phthalimidomethyl phosphorodithioate, Stauffer Chemical Company) is a highly effective scabicide against P. ovis, when employed once only in a dipping vat, at a concentration of 0.25% aqueous emulsion. The product is satisfactory from the standpoint of stability in the dipping vat; it is not toxic to the host, and presents no tissue residue problems.

Equally effective biologically, but less satisfactory from either host toxicity or tissue residue aspects or both, are malathion and coumaphos (Co-Ral).

Two drugs awaiting final evaluation are ciodrin (Cio-Rid) and diazinon, which appear biologically effective at present. Ciodrin, in the formulation used, like Prolate, is acceptable for application to dairy cattle, since its application to livestock is devoid of residue retention in milk and edible tissues.

Transmission of Psoroptes ovis by animate vectors

On the station premises, P. ovis, the common scabies mite, sometimes spreads, unaccountably, from infested flocks to clean groups of sheep. To determine if blackbirds could act as mechanical agents in spreading mites between groups of sheep, an experiment was initiated in the spring of 1967.

During the spring migration of blackbirds through central New Mexico, 4 redwings and 3 cowbirds were trapped and confined to a 20' x 40' building containing 1 group of scabby sheep and 1 group of clean sheep, housed in separate pens. Each pen was 12' x 14' in size, and contained 6 sheep. The pens were separated in the building by 16' of clear floor space. The birds were unhampered in their movement within the building, and ate and drank with the sheep in either pen.

The sheep and birds have shared the same accommodations from March 20 to June 30, a total of 103 days, without any detectable transfer of mites from the infested group to the noninfested group of sheep.

Host-parasite interactions between sheep and the common scabies mite, Psoroptes ovis

At the Parasite Research Laboratory, Albuquerque, N. M., when sheep carrying P. ovis infestations were individually isolated, self-cure frequently resulted. This phenomenon invariably took place, in an average of 10 months, when the hosts were infested with avirulent, or benign strains of P. ovis. It is assumed that immune responses on the part of the host were responsible for this phenomenon.

Also, as mite populations on individually isolated hosts neared extinction, newly introduced, uninfested recipient hosts quickly acquired infestations. Thereafter, thriving infestations on the donor hosts would be reestablished from the recipient host in contact with it. This observation pointed strongly to a host immune response of very short duration, probably in the nature of a localized skin cellbound antibody, and not a circulating antibody.

To demonstrate further that host resistance (rather than possible strain exhaustion because of lethal genetic factors within an isolated mite population) accounted for recovery from scabies, the experiment was established on a flock basis. It is assumed that the self-cure phenomenon will occur in a closed flock just as effectively as on an individual host, if strain exhaustion is the factor responsible.

(Albuquerque, New Mexico) (ADP b3-25)

G. Biology and Control of the Liver Fluke, *Fasciola hepatica*, in the Southwest

The common liver fluke is a cause of considerable monetary loss to cattle and sheep producers in northern New Mexico, southern Colorado, and eastern Arizona. Recent investigations carried out on farms and ranches in these areas and in the Parasite Research Laboratory at Las Cruces, New Mexico, showed for the first time the identities of at least some of the snails that transmit liver flukes in these localities. This information may help control the parasite through the control or eradication of the snail vectors either by biological or chemical means. The control of liver flukes also may be attained by using drugs that will kill and remove the flukes from the host animals. Several drugs will remove the adult stages but few are effective against the young flukes. Several drugs were evaluated and Bayer 9015 and Tremerad were the most promising. Sulfoxide of bithionol is effective but may be too toxic at the required dosage.

(Las Cruces, New Mexico) (ADP b3-27)

H. Biology and Control of Worm Parasites of Sheep in the Southwest with Emphasis on Immunization Procedures for the Control of Haemonchosis

It has been confirmed that the capability of lambs to develop immunity to haemonchosis, one of the most serious of the parasitic diseases, increases with age. This information was obtained by comparing lambs 3 and 6 months of age. The procedure was to inoculate with an attenuated strain of the stomach worm from pronghorn antelope, then to remove the immunizing infection with a drug. This step was followed by challenge with sheep strain stomach worms to determine the degree of immunity. This information may be used to an advantage by sheep producers by limiting the grazing and consequent exposure to infection of younger lambs.

Studies of the protein patterns of lambs immunized against stomach worms were carried out to define any correlation between the patterns and the degree of immunity. Tests were made with serum and stomach wall extracts. In general, immunization procedures resulted in an increase in gamma globulin and a decrease in albumin but these changes were not directly proportional to the degree of immunity. Patterns of stomach wall extracts were sufficiently different from serum patterns to warrant further study. These findings increase the store of knowledge needed to find an effective and practical method of immunizing lambs and calves on the farm or ranch.

In order to alleviate the losses suffered by western sheep producers from the condemnation of livers containing fringed tapeworms, we continued our search for a drug capable of removing the parasites. Five compounds were investigated in tests involving 27 infected animals. Bithionol sulfoxide was the most effective compound. This work will be continued during the coming year.

(Las Cruces, New Mexico) (ADP b3-28)

I. Immunological Response to Strongyloidiasis

A P.L. 480 Grant was executed during the reporting year with the University of Warsaw, Warsaw, Poland, for research on strongyloidiasis.

Strongyloides papillosus is a very minute nematode occurring in the small intestine of sheep, goats, and cattle in all parts of the world. It is like the hookworms in that the infective larvae can enter the host either by ingestion or penetrating the skin. This parasite is also unusual in that it has a parasitic adult phase and sometimes a free-living sexual phase. The parasitic adults are all female in form. They are parthenogenetic and their eggs may give rise, outside the host, directly to infective larvae of another parasitic generation or to a free-living generation of minute males and females.

(Warsaw, Poland) (E21-ADP-12)

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AREA NO. 14 -- PARASITES AND PARASITIC DISEASES OF POULTRY

Problem. Parasites and parasitic diseases probably cost the poultry industry many millions of dollars annually by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices, intended to avoid spread of parasites and to control them, have been ineffectual as shown by the increasing importance of certain parasites in broiler production. The problem is to develop--through a planned, balanced program of basic and applied research--methods for preventing, controlling, or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 4.0 scientist man-years. This effort is applied as follows:

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biological Investigations of Protozoan Parasites and Parasitic Diseases, with Special Reference to Those of the Gastrointestinal Tract 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and under cooperative agreement with the Agricultural Experiment Station, College Station, Texas.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 4.6 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Biological Investigations of Protozoan Parasites and Parasitic Diseases of Poultry

Blackhead, or histomoniasis, is a parasitic disease of turkeys, chickens, and many common game birds. It costs the poultry growers of the United States about \$10 million a year. It is caused by a microscopic, 1-celled parasite, Histomonas, yet is carried by a small parasitic worm, the cecal worm, or Heterakis, that also lives in turkeys, chickens, and some game birds. Cecal worms can become parasitized by the blackhead parasite, Histomonas. Infections of the cecal worm can, in turn, be spread by earthworms, which are often abundant in the soil of poultry yards and turkey ranges, and may be eaten by these birds. When the earthworms contain young cecal worms with blackhead parasites, the birds that eat such earthworms may develop blackhead.

Our studies have been directed at the host and all 3 types of intermediate hosts involved in the disease and its transmission--the histomonad, the cecal worm, and the earthworm.

One set of studies concerns learning the nature of the blackhead parasite in the absence of any of its hosts. For these studies, the histomonads are grown in test tubes in the laboratory. Under these conditions, the parasite slowly changes. It first loses its ability to cause disease in birds, but still retains some of its ability to call forth immune responses in the bird. Later, it completely loses its ability to incite immune response, but it will still grow harmlessly in birds. Still later, it loses even its ability to grow in birds, and apparently is completely adapted to life in the test tube.

Another set of studies has concerned the growth of the cecal worm, both in the presence and absence of the histomonad. Chickens and turkeys developed some immunity to the cecal worm, but this immunity was not sufficiently complete to control entirely the worm's ability to transmit the blackhead organism.

A third set of studies dealt with the role of earthworms in transmitting both the cecal worm and the histomonad. Because earthworm activity is so closely related to climatic and soil conditions, and seasonal changes, these studies are continuing. However, when these conditions are considered, we can now use the earthworm as a means of estimating the extent to which soil is contaminated with infective cecal worms and histomonads. Using this method, poultry growers may avoid costly mistakes in the use of range land or poultry yards.

In still a fourth series of tests, we have demonstrated differences in the susceptibility of birds of different species (chickens, turkeys. etc.) and of different breeds of 1 species (New Hampshire, White Leghorn, etc.) to histomonads and cecal worms. Therefore, soil relatively safe for use by some birds would be hazardous for use by others.

(Beltsville, Maryland) (ADP b4-11)

In the cooperative research at the Texas Agricultural Experiment Station, College Station, Texas, a specific Histomonas meleagridis antiserum was produced in rabbits, conjugated with fluorescein, and successfully used to identify histomonads in cell culture fluids and experimentally-infected turkeys. Attempts are now being made to use the system in making biological studies of histomonads in transport hosts.

(College Station, Texas) (ADP b4-11)

Under a P.L. 480 Grant to the National Taiwan University, Taipei, Taiwan, China, studies are being conducted on leucocytozoonosis of chickens. The natural occurrence of this disease was rare from November to April. The studies were concerned with the mode of transmission, pathology, the habitation places of the intermediate host, as well as the prevention of leucocytozoonosis by chemicals.

It has been proved that Leucocytozoon sabrazesi propagates chiefly in rice fields.

(Taipei, Taiwan, China) (A6-ADP-1)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Protozoan Parasites

Krishnamurti, P. V. 1967. Cultivation of Histomonas meleagridis free of bacteria. Doctoral Dissertation - Texas A&M Univ.

Lund, E. E., Augustine, P. C., and Ellis, D. J. 1966. Immunizing action of in vitro attenuated Histomonas meleagridis in chickens and turkeys. Exp. Parasitol. 18:403-407.

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coturnix japonica, as a host for Heterakis and Histomonas. Laboratory
Animal Care 17:110-113.

AREA NO. 15 - TREATMENT FOR REMOVAL OF PARASITES
OF DOMESTIC ANIMALS'

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability, few, if any, of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research, control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 9.5 scientist man-years. This effort is applied as follows:

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 2.0 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Evaluation and Standardization of Antiparasitics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Control of Lice on Cattle 1.5 at the Albuquerque, New Mexico, field station.

PROGRAM OF STATE EXPERIMENT STATIONS

Research of the State stations in this area is included in Areas 11, 12, 13, 14, and 16.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Investigations to Develop New and Improved Chemical Agents for the Treatment, Prevention, or Control of Helminthic Parasites in Farm Animals

At the Beltsville Parasitological Laboratory, researchers found that tetramisole (dl 2, 3, 5, 6-tetrahydro-6-phenylimadizo (2,1-b) thiazol hydrochloride), a new anthelmintic for livestock, was effective against adult stages of the large stomach worm, Haemonchus contortus, and the stomach and intestinal hairworms, Trichostrongylus axei and T. colubriformis, respectively, of sheep and goats. These roundworms interfere with the normal metabolism of the host, resulting in morbidity and sometimes death. Preliminary data indicate that tetramisole compares favorably with thiabendazole, and purified, micronized phenothiazine as effective treatment against these 3 major parasitic pathogens of ruminants.

Tetramizole shows promise as a practical chemotherapeutic agent against the nodular worm, Oesophagostomum dentatum, of swine. Two forms of the drug, namely, l-(levo) and dl-(dextro-levo)tetramisole, were given admixed with feed at dose rates of 8 and 15 mg./kg., respectively. Optically, the active l-tetramisole had better anthelmintic activity at half the dose rate of the optically inactive racemic dl- form of the drug.

Thiabendazole was apparently effective against the kidney worm, Stephanurus dentatus, of swine. A mature Hampshire sow with a patent kidney worm infection was given thiabendazole admixed with feed for 5 consecutive days at the rate of 50 mg./kg. of body weight. Kidney worm eggs disappeared from the urine and have not been detected during a post-treatment holding period of more than 1 month. Periodic examinations are being continued.

(Beltsville, Maryland) (ADP b5-18)

B. Investigations of Dimetridazole and Other Potential Chemotherapeutic Agents as Treatments for Bovine Venereal Trichomoniasis

In research work at the Beltsville Parasitological Laboratory, Tri-trichomonas foetus infections were eliminated from 2 cows by single intravenous injections of 100 mg./kg. dimetridazole per kilogram of body weight. One cow was freed of a T. foetus infection by oral administration of dimetridazole at 50 mg./kg. daily for 5 successive days. The infection

persisted in another animal that received the same oral dosage.

A dimetridazole-resistant strain of T. foetus experimentally established in hamsters for over 3 years retains its original level of tolerance for the chemical.

(Beltsville, Maryland) (ADP b5-19)

C. Evaluation, Development, and Standardization of Antiparasitics

At the Beltsville Parasitological Laboratory researchers found that a strain of Eimeria tenella developed a pronounced tolerance for Novostat after repeated passage through chickens fed mash containing the coccidiostat.

Serially propagating an amprolium-resistant strain of E. tenella in unmedicated chickens for 10 generations did not increase the strain's sensitivity to the coccidiostat.

An amprolium-resistant strain of E. tenella serially propagated in chickens fed mash containing acriflavine regained their sensitivity to amprolium.

Thiabendazole had effective action against the nodular worm, Oesophagostomum dentatum, of swine. When given admixed with the feed at dose rates of 50 and 100 mg./kg. of body weight, the drug was 85% effective at the lower dose rate and removed all worms at the 100-mg. level.

(Beltsville, Maryland) (ADP b5-20)

The Experiment, Georgia, substation of the Regional Animal Disease Research Laboratory reported as follows on the results of evaluation, development, and standardization of chemical methods of established or reported value for controlling parasitic diseases of livestock and poultry.

An experiment to evaluate the efficacy of thiabendazole, when given as a feed additive in a low level dosage rate to control internal parasites of cattle has been concluded. Daily doses of 1 and 4 mg./kg. of body weight apparently reduced the number of nematode eggs produced and increased the average daily gain of the cattle. Also, the cattle from the treated groups harbored fewer worms than those from the control group.

(Experiment, Georgia) (ADP b5-20)

Also, the efficacy of Co-Ral (0,0-diethyl 0-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate) was tested at the above station as an anthelmintic in cattle. Critical tests using 4 steers indicated that when administered as a feed additive at a dose level of 1.25 mg./kg. of body weight for 6 consecutive days, Co-Ral was highly effective against mature

Haemonchus placei, Cooperia punctata, and C. oncophora, and somewhat less effective against Ostertagia ostertagi, Trichostrongylus axei and T. colubriformis. It was apparently ineffective against Bunostomum phlebotomum, Oesophagostomum radiatum, Trichuris spp., Moniezia spp., and larvae of O. ostertagi. No signs of toxicity occurred in any of the steers treated.

(Experiment, Georgia) (ADP b5-20)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Control of Parasitic Diseases of Livestock and Poultry

Ciordia, Honorico, and Baird, D. M. 1967. Anthelmintic efficacy of low level dosages of Co-Ral administered as a feed additive to cattle. Georgia Vet. 19:9-11.

Colglazier, Merle L., Wehr, Everett E., Burtner, Roy H. and Wiest, Louis M. 1967. Haloxon as an anthelmintic against the cropworm Capillaria contorta in quail. Avian Dis. 11:257-260.

McLoughlin, D. K. 1966. Observations on the treatment of Trichomonas gallinae in pigeons. Avian Dis. 10:288-290.

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Wehr, E. E., Colglazier, M. L., Burtner, R. H., and Wiest, L. M., Jr. 1967. Methyridine, an effective anthelmintic for intestinal threadworm, Capillaria obsignata, in pigeons. Avian Dis. 11:322-326.

AREA 16 - MISCELLANEOUS PARASITES AND PARASITIC DISEASES

Problem. Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wildlife. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, microbiologists, and veterinarians engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 11.5 scientist man-years. This effort is divided among subheadings as follows:

Publication and Maintenance of Author, Subject, and Host Index-Catalogues 2.0 at the Beltsville Parasitological Laboratory.

Immunologic and Other Biologic Approaches to the Prevention and Control of Parasitic Diseases 1.7 at the Beltsville Parasitological Laboratory and under cooperative agreement with the College of Veterinary Medicine, University of Minnesota, St. Paul, and Wisconsin Agricultural Experiment Station, Madison.

Chemical and Physical Elements of Parasites and Parasite-Host Relationship 2.0 at the Beltsville Parasitological Laboratory.

Taxonomic Investigations and Identification of Parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of Parasite Collection 0.3 at the Beltsville Parasitological Laboratory.

Pigments of Parasites 0.5 at the Beltsville Parasitological Laboratory.

Biology, Epidemiology, and Pathogenicity of Demodectic Mange 1.0 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Cytological Investigations of Protozoan Parasites That Penetrate the Gastro-intestinal Tract of Poultry and Other Farm Animals 2.0 at the Beltsville Parasitological Laboratory.

PROGRAM OF STATE EXPERIMENT STATIONS

The research effort of the State experiment stations in this area totals 2.2 scientist man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Publication and Maintenance of Author, Parasite-Subject, and Host Catalogues of the Index-Catalogue of Medical and Veterinary Zoology.

In preparation for publication: Material for Supplement 16: Part 5, Arthropoda, etc.; Part 6, Subject-Headings-Treatment; Part 7, Hosts; Supplement 17: Part 1, Authors; Part 2, Protozoa; Part 3, Trematoda and Cestoda; Part 4, Nematoda and Acanthocephala; Part 5, Arthropods, etc.; Part 6, Subject-Headings-Treatment; Part 7, Hosts; and for the Subjects: Trematoda and Trematode Diseases, Part 7, Supergenera and Genera Q-Z; and Part 8, Hosts.

In press: Supplement 16: Part 3, Trematoda-Cestoda; Part 4, Nematoda and Acanthocephala; and the Subjects: Trematoda and Trematode Diseases, Part 6, Supergenera and Genera N-P.

Published in late 1966: The Host section of Supplement 15, and the Authors A-Z, and Parasite-Subject Catalogue: Protozoa of Supplement 16.

(Beltsville, Maryland) (ADP b6-9)

The Index-Catalogue of Medical and Veterinary Zoology has been maintained and expanded in its various sections: Author, Parasite-Subject, and Host Catalogues, and Check List of Specific and Subspecific Names. New entries augmenting the Catalogues are as follows: Author entries, 7,870; Parasite-Subject entries, 25,211 (including 21,170 parasite entries, 1,838 anthelmintic entries, 2,203 subject-heading entries); and Host entries, 8,745. New genera and species of parasites are as follows: Protozoa, 18 n.g., 184 n.sp.; Trematoda, 31, n.g., 196 n.sp.; Cestoda, 17 n.g., 60 n.sp.; Nematoda, 44 n.g., 565 n.sp.; Arthropoda, 53 n.g., 440 n.sp. There have been 92 new citations of periodicals added to the Catalogue.

Two bibliographies, covering current parasitological literature, have been received from the National Library of Medicine that were compiled by the computer, Medical Literature Analysis and Retrieval System (MEDLARS). These bibliographies and other material are being indexed. An average of 500 incoming periodicals were examined each day at the National Agricultural Library for parasitological papers.

The Index-Catalogue has had more than 138 visitors from the United States and 5 other countries, some of them staying several days and consulting the Catalogue as a source of information.

(Beltsville, Maryland) (ADP b6-14)

B. Investigation of Immunologic and Other Biologic Approaches to the Prevention and Control of Parasitic Diseases

At the Beltsville Parasitological Laboratory, the life cycle of Ascaris lumbricoides was restudied in the normal swine host and differed in important respects from that previously reported. Certain morphological features previously unknown, or inadequately described, characterized the developmental stages. The location and rate of development of larvae up to 4th stage were determined for the 1st time wholly from the normal host.

To help understand the ways by which coccidial parasites infect the host, a method was devised for readily stopping their developmental processes. The method involves obtaining, by necropsy, the intestinal contents which contain the parasites and storing them at a temperature slightly above freezing; rapid cooling is essential. The low temperatures stop the parasite development. At any time thereafter, during a period of several hours, development can be restored at will by warming the material to the temperature of the host's body. This procedure for the 1st time provides a method by which morphological as well as other studies can be made on various developmental stages of the parasites. The method has also been useful in classroom work in some educational institutions.

(Beltsville, Maryland) (ADP b6-10)

In cooperative research at the University of Minnesota, studies were continued on the helminth parasites of cattle, sheep, and poultry.

Studies on the large American liver fluke (Fascioloides magna) have progressed slowly with the establishment of a laboratory colony of potential intermediate hosts to supply infective fluke forms (metacercariae). Stagnicola species of snails have been infected with F. magna and have yielded a moderate supply of metacercariae. The latter have been used in preliminary trials to demonstrate the experimental production of Black disease (necrotic hepatitis) using rabbits as the host animal. To date, results have been inconclusive.

In the work on blackhead (Histomoniasis), attempts to isolate the organism (Histomonas meleagridis) from chickens and turkeys have been in progress. Isolations have been made in about 20% of the attempts: those birds with soft, semifluid, blood-tinged cecal content appear to have the highest percentage of recovery.

In vitro serial culture of H. meleagridis has been made and maintained for 5 transfers.

In studies on the cecal worm (Heterakis gallinae), major efforts have been directed toward establishing a culture of pathogen-free Heterakis gallinae (i.e. Histomonas-free). This work has involved the use of a protozoacide at time of infection of birds with Heterakis ova; however, this procedure did not prove satisfactory. The use of unusual hosts was also adopted as a possible screening mechanism, but this method was equally unsatisfactory. Ova of H. gallinae considered free of Histomonas were obtained from a pheasant source in England. At the present time, the establishment of this strain in chickens is being accomplished.

(St. Paul, Minnesota) (ADP b6-10)

At the University of Wisconsin, Department of Veterinary Science, the studies were concerned with isolation of relatively nonpathogenic populations of the important worm parasite of sheep, Haemonchus contortus, with the aim of developing a vaccine. In the first 2 years of the study, 53 collections were made from widely separated areas in the U.S.; 26 populations were grown successfully in seed lambs, 17 have been studied intensively, and 9 more are in current intensive evaluation. The project has been outstandingly successful. Three relatively nonpathogenic populations have been found. More collections will be made, and tests are underway to mark Haemonchus populations with radioactive material. It is hoped that the ability of the nonpathogenic populations to compete with normal populations can be studied late in 1967-68. The success to date indicates that continued cooperative support will be sought.

(Madison, Wisconsin) (ADP b6-10)

C. Chemical and Physical Elements of Parasites and Parasite-Relationships

The antigens present in the excretory gland of the swine kidney worm, Stephanurus dentatus, have been further separated. Using gel filtration, we separated the specific antigens from the nonspecific (i.e. the specific antigens react only with serums from kidney worm-infected swine whereas the nonspecific antigens react both with serums from infected and normal swine). The nonspecific antigens were separated from each other by preparative disc electrophoresis.

A structure similar to the "cytostome" of Plasmodium was observed by electron microscopy in a high proportion of Babesia equi. Methodology of orienting small nematodes (e.g. early larval stages) for embedding and sectioning has been worked out, and accurate, 1 μ cross-sections can now be cut for observations.

(Beltsville, Maryland) (ADP b6-11)

D. Taxonomic Investigations and Identification of Helminths and Other Parasites

Work was continued on the species of thread-necked strongyles of North American ruminants. Six species parasitized domestic sheep in the United States. They are, in descending order of their incidence, Nematodirus spathiger, N. abnormalis, N. filicollis, N. lanceolatus, N. helvetianus, and N. davtiani. These species have been confused with one another in earlier publications and a key with illustrations was prepared for their easy identification. Nematodirus abnormalis was previously considered relatively uncommon in sheep and N. helvetianus, a cattle parasite, had not previously been reported in sheep in this country. A description of a deer parasite, N. odocoilei was prepared and published. This species now occurs in deer in British Columbia, Georgia, Montana, Oregon, and Wyoming, and in elk in Michigan.

Studies were completed on other parasites of ruminants. Detailed illustrative redescrptions were prepared of 2 medium stomach worms that have not previously been morphologically clearly defined. Ostertagia bisonis, originally described from bison in Canada, has been mistakenly called O. bellae nomen nudum and O. orloffii in American literature. This parasite has been associated with clinical parasitism in cattle and also occurs in antelope, deer, bighorn sheep, and Barbary sheep. Ostertagia mossi, originally described from deer in Pennsylvania, was incorrectly redescribed in 1956 (Lai) from specimens of another species from cattle and goats in Sardinia. Although it has also been reported in West Pakistan and the Netherlands, the only host and localities where it occurs with certainty here are in deer

in Pennsylvania, New York, and Georgia. Two nematodes not heretofore known to occur in domestic sheep in the United States, Cooperia surnabada, a parasite of cattle, and Hyostrongylus rubidus, a common parasite of swine, were among nematodes of sheep origin from Illinois. Two Cooperias of sheep, C. punctata and C. spatulata, which are morphologically very similar, were compared and their diagnostic characteristics illustrated as an aid in their identification.

Another taxonomic work completed is a description of a new species of Capillaria from the intestine of a man who died of clinical parasitism in the Philippines. This parasite represents the first case of intestinal capillariasis of man. Additional clinical cases have recently been diagnosed in the Philippines. A new genus and species of a spiruroid nematode was described from the pancreatic ducts of a white-tailed marmoset. This nematode is peculiar in having characteristics of several groups which make it difficult to classify. Several hosts, imported from Brazil for research in Texas, were infected.

(Beltsville, Maryland) (ADP b6-12)

At the Beltsville Parasitological Laboratory, 547 lots of specimens were identified (trematodes 33, cestodes 28, nematodes 449, acanthocephalans 26, and arthropods 11). The nematodes were collected from a large variety of hosts and represent numerous different species. Among these were large collections from wild ruminants in South Dakota, elk in Michigan, primates imported and reared for research, market fish, marine mammals, and numerous parasites in tissue sections on slides. Several of the parasites represent new host and distribution records. Many of the specimens were tentatively identified by the sender and were sent for concurrence of identification or to determine if they were new to science. Considerable time was spent with visiting scientists who requested assistance in the identification of parasites collected from animals used in their search. They are employed by the following agencies, institutions, or universities: Armed Forces Institute of Pathology, Edgewood Arsenal, Fort Detrick, Animal Health (USDA), Plant Nematology (USDA), Meat Inspection (USDA), U. S. Fish and Wildlife Service, Southwest Foundation for Research and Education, University of Georgia, University of Montana, Bowman Gray Medical School, and Dental Science Institute of Houston, Texas.

The records of all the ticks identified that were from imports in the United States and at ports of entry were summarized for publication. The ticks were removed from cattle, horses, numerous kinds of zoo animals, beef, cattle hides, a snake in a shipment of bananas, palm leaves, mail bags, medicinal herbs, bird guano, and hair, from various parts of the world. They represented 13 species of Amblyomma, 3 of Boophilus, 4 of Dermacentor, 1 of

Haemaphysalis, 6 of Hyalomma, 3 of Ixodes, 5 of Rhipicephalus, and 2 species of argasids. This study revealed 3 important points: 1) Most of the exotic ticks that are found on imports are males; females apparently drop off at foreign quarantine stations or en route; 2) native as well as exotic ticks on imports may be vectors of foreign diseases, e.g. introduction of the tropical horse tick with equine piroplasmosis in Florida; and 3) harmful ticks may occur on various items and on abnormal hosts, e.g. cattle fever ticks on beef, hides, and hair, and cattle fever ticks and tropical horse ticks on an ocelot.

(Beltsville, Maryland) (ADP b6-16)

E. Maintenance of Parasite Collection

There were 850 lots of specimens (protozoans 17, trematodes 170, cestodes 45, acanthocephalans 52, nematodes 552, arthropods 12, and miscellaneous 2) added to the parasite collection. These include many type specimens of new genera and species, as well as 1 relatively large collection of parasites of monkeys and other primates which are of considerable value because of the increased use of these animals in research. During the year, 212 inquiries concerning the deposit or loan of specimens were answered. Specimens of Nematodirus of sheep origin in 130 lots were studied and numerous specific names were redetermined.

(Beltsville, Maryland) (ADP b6-15)

F. Pigments of Parasites

The hemoglobin of Syngamus trachea, a parasite of turkeys, binds oxygen less tenaciously than the hemoglobins of many other parasitic nematodes. However, it binds oxygen more tenaciously than the hemoglobin of the turkey host. The S. trachea hemoglobin has a slightly lower molecular weight and higher percentage of iron than the hemoglobin of the host.

(Beltsville, Maryland) (ADP b6-17)

G. Biology, Epidemiology, and Pathogenicity of Demodectic Mange of Domestic Livestock

Demodex spp. have been reported from the various hosts and from various tissues of these hosts. An attempt has been made to study the incidence of Demodex spp. in the various livestock hosts and other experimental animals and to study the incidence in the various tissues of the body.

Eyelids, noses, lips, lymph glands, and other parts of the skin obtained from slaughterhouses or from experimental animals are placed in a beaker of

warm or hot 10% KOH to digest. After the material has digested, the mixture is poured into 15-ml. centrifuge tubes and centrifuged at 1200 r.p.m. for 3 minutes. The supernatant is poured off, 10 ml. of a sugar solution containing 7 parts Karo syrup and 1 part water is added, and the contents of the tube stirred with a glass rod. Enough additional sugar solution is added to bring the surface of the liquid barely above the rim of the tube, and a coverslip is placed thereon in contact with the liquid. After centrifuging for 3 minutes at 1000 r.p.m., the coverslip is removed, placed on a slide, and examined microscopically under low power for the presence of Demodex spp. The level of the liquid is brought up again above the rim of the tube by adding more sugar solution and also another coverslip, centrifuging at 1000 r.p.m. for 3 minutes, removing coverslip, placing on a slide, and examining for Demodex spp. (masceration-flotation technique). The second coverslip is essential since any mites present may not come up on the first coverslip. Data were recorded as to type of specimen, location of specimen, host, procedure, and results.

The incidence of Demodex spp. in the eyelids of horses, obtained from the Indian reservations of New Mexico and Arizona, is believed to be the highest recorded. The eyelids were taken from horses that had no clinical signs of Demodex infestation.

The incidence of demodectic mange, which can be recognized by the presence of skin lesions in cattle, is much lower in New Mexico than in other regions of the country. However, a random sampling of native cattle suggests an appreciable incidence of inapparent parasitism. This is also true of native horses and swine.

No demodectic infestations occurred in the young of any livestock except goats. Dairy goat breeds provide the best subjects for use in all aspects of investigations into domestic mange.

(Albuquerque, New Mexico) (ADP b6-18)

H. Cytological Investigations of Protozoan Parasites that Penetrate the Gastrointestinal Tract of Poultry and Other Farm Animals

Excysted sporozoites of Eimeria meleagriditis, E. necatrix, and E. acervulina penetrated cells in monolayer cultures of embryonic bovine, embryonic human, ovine, and porcine kidney. However, E. meleagriditis developed only in bovine cultures. Eimeria necatrix also developed to mature 1st generation schizonts in embryonic bovine kidney (serial cultures) and to immature schizonts in serial cultures of porcine kidney. Neither of these 2 species developed in the 5th and 18th passages of ovine kidney or in the 45th and 52nd passages of human kidney. Eimeria acervulina and E. gallopavonis did

not develop in any of the cell cultures inoculated. Studies indicate that species differ in the inherent mechanism that must be activated before development will take place. They also indicate that a cellular activating factor, when inherently present, becomes progressively more pronounced through serial passage. Findings also suggest that cellular resistance to infection is lost during serial passage and progressively more of a cellular activating factor, perhaps part of a resistance complex, is made available.

Eimeria acervulina, E. necatrix, E. meleagritidis, and E. gallopavonis coccidia, which parasitized the small intestine of chickens and turkeys, used the glycogen reserves within sporozoites during excystation and subsequent penetration of the cells in monolayer cultures of serial embryonic bovine kidney. The oxygen consumption rate of sporozoites increased during this interval, and cytochemical determinations revealed no acid nor alkaline phosphatase activity.

Cytochemical studies demonstrated that macrophages in embryonic chicken intestinal epithelial cell cultures phagocytized oocysts, sporocysts, and sporozoites that were inoculated into the cultures.

The number of sporozoites of the turkey coccidium, Eimeria meleagritidis, did not alter the amount of cytoplasmic acid phosphatase of parasitized cells in monolayer cell cultures as with intracellular bacteria. This finding indicates a difference in cell response to coccidia.

New methods for sporulation and purification of coccidian oocysts have been developed. Pure oocysts with better than 95% sporulation rate can now be produced with a saving of 29 man hours per liter of fecal suspension.

A new species of Cryptosporidium was established as the parasite in the small intestine of the guinea pigs in the colony of the Walter Reed Army Institute of Research (WRAIR). In cooperation with the Experimental Pathology Division of WRAIR, experimental transmission was carried out and the host specificity of the new species established. The ultrastructure of the tissue stages of this coccidium was studied and the membrane association between the coccidium and the host cell established.

(Beltsville, Maryland) (ADP b6-19)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Publication of Index-Catalogues

Humphrey, Judith M., and Segal, Dorothy B., with the assistance of Beard, Mary I., Edwards, Shirley J., and Kirby, Margie D. 1966. Index-Catalogue of Medical and Veterinary Zoology, Supplement 15, Hosts, U. S. Government Printing Office.

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			Summary of Progress	Area & Sub- heading
ADP a1	Infectious and noninfectious diseases of cattle			
ADP a1-9 (R) *	Diagnosis of bovine vibriosis by immuno-fluorescent methods	Ames, Iowa	No	
ADP a1-13 (R)	Tuberculosis of cattle	Ithaca, N.Y.	Yes	1-A
ADP a1-14 (C),(R)*	Mucosal-respiratory disease-complex of cattle	Ames, Iowa	Yes	1-B
		East Lansing, Mich.	Yes	1-B
	Bovine virus diarrhea	Ames, Iowa	Yes	1-C
ADP a1-15 (R)	Mastitis of cattle	Ames, Iowa	Yes	1-C
		Ames, Iowa	Yes	1-D
		Davis, Calif.	Yes	1-D
ADP a1-21 (R)	Epizootic bovine abortion	Ames, Iowa	No	
		Davis, Calif.	Yes	1-E
ADP a1-22	Investigations of foot rot (infectious pododermatitis) of cattle	Ames, Iowa	No	
ADP a1-24 *	Etiologic, cytologic, and histochemic studies of pulmonary adenomatosis in cattle	Ames, Iowa	Yes	1-F
ADP a1-25	Immunization against bovine leptospirosis	Ames, Iowa	Yes	1-G
ADP a1-26	Chemotherapy in leptospirosis disease of cattle and swine	Ames, Iowa	Yes	1-G
ADP a1-28	Physiopathologic aspects of <u>Lupinus sericeus</u> and <u>Lupinus caudatus</u> plants on livestock	Logan, Utah	Yes	3-F
ADP a1-29 (C)	Enteritis in young calves	Moscow, Idaho	Yes	1-H
ADP a1-30	Bovine lymphosarcoma	Ames, Iowa	Yes	1-I
		Lincoln, Nebr.	Yes	1-I
		Ithaca, N.Y.	Yes	1-I
ADP a1-31	Characterization of factors affecting the proliferation of <u>Pasteurella</u> sp. in the host	Ames, Iowa	Yes	1-J
ADP a1-32	Characterization and classification of members of the genus <u>Brucella</u>	Ames, Iowa	Yes	1-K
		St. Paul, Minn.	Yes	1-K
		Wooster, Ohio	Yes	1-K
		Madison, Wisc.	Yes	1-K
ADP a1-33	The effect of <u>Mycoplasma</u> on bovine reproduction	Ames, Iowa	Yes	1-M
ADP a1-35	Paratuberculosis (Johne's disease) of cattle	Ames, Iowa	Yes	1-L
ADP a1-37	Pink-eye (infectious keratitis) of cattle	Ames, Iowa	No	
ADP a1-38 (C)	Investigations to develop in vitro cytotoxic procedures for study and detection of tuberculosis sensitivity in cattle	East Lansing, Mich.	Yes	1-B
ADP a1-39	Pathogenesis of <u>Pasteurella</u> pneumonia	Ames, Iowa	Yes	1-J
ADP a1-40	Immunogenic and bacteriologic studies of <u>Vibrio fetus</u> in cattle	Ames, Iowa	Yes	1-A
ADP a1-41**	Mechanisms of the virulence of leptospirosis of cattle	Ames, Iowa	Yes	1-G
ADP a1-42**	Investigations of enteric diseases of cattle	Ames, Iowa	Yes	1-C
ADP a2	Infectious and Noninfectious diseases of swine			
ADP a2-15 (R)	Erysipelas of swine	Ames, Iowa	Yes	2-B
ADP a2-16 (R)	Brucellosis of swine	Ames, Iowa	Yes	2-C
ADP a2-17 (C)	Hog cholera	Ames, Iowa	Yes	2-A
		Lincoln, Nebr.	No	
ADP a2-19	Abscesses in swine	Ames, Iowa	Yes	2-D
		Ft. Collins, Colo.	Yes	2-D
		Lafayette, Ind.	No	

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			Summary of Progress	Area & Sub- heading
ADP a2-20	Etiology of atrophic rhinitis in swine	Ames, Iowa	Yes	2-E
ADP a2-21	Pathogenesis of swine erysipelas	Ames, Iowa	Yes	2-B
ADP a2-22	Effect of antiviral drugs on viruses associated with transmissible gastro- enteritis	Ames, Iowa	No	
ADP a2-23	Characterization of viruses associated with transmissible gastroenteritis	Ames, Iowa Davis, Calif. Lafayette, Ind.	No Yes Yes	2-F 2-F
ADP a2-24 **	Evaluative studies of recommended procedures for final stages of hog cholera eradication program	Ames, Iowa	No	
ADP a3	<u>Infectious and noninfectious diseases of sheep and goats</u>			
ADP a3-4 (R)	Viral ulcerative dermatosis of sheep	Ft. Collins, Colo.	Yes	3-D
ADP a3-5*	Bluetongue in sheep - diagnosis, trans- mission and control	Denver, Colo.	Yes	3-A
ADP a3-6	Paratuberculosis (Johne's disease) of sheep and goats	Ames, Iowa	Yes	3-C
ADP a3-7 (R)	Toxicological effects of oxalate-containing plants on livestock	Logan, Utah	Yes	3-E
ADP a3-8*	Identification of teratogenic agent in <u>Veratrum californicum</u>	Logan, Utah	Yes	7-0
ADP a3-9*	Chronic toxicity of herbicide accumulation in sheep tissues	Logan, Utah	Yes	7-Z
ADP a3-11	Metabolic, antigenic, and pathogenic characteristics of <u>Vibrio fetus</u> of sheep	Ames, Iowa Ft. Collins, Colo. Bozeman, Mont. Logan, Utah Greenport, N.Y.	No Yes Yes Yes Yes	3-B 3-B 3-B 3-B 3-C
ADP a3-12	Immunology of scrapie			
ADP a3-13**	Cytopathology of bluetongue and vesicular stomatitis viruses in salivary glands of insect vectors	Denver, Colo.	Yes	3-A
ADP a3-14**	Reactions for bluetongue and vesicular stomatitis viruses	Denver, Colo.	Yes	3-A
ADP a3-15**	Physico-chemical and morphological characterization of bluetongue and vesicular stomatitis viruses	Denver, Colo.	Yes	3-A
ADP a3-16**	Role of <u>Culicoides variipennis</u> and other insect species in the transmission of vesicular stomatitis and bluetongue virus	Denver, Colo.	Yes	3-A
ADP a3-17**	Fate of bluetongue and vesicular stomatitis viruses in experimental hosts	Denver, Colo.	Yes	3-A
ADP a3-18 **	Clinicopathologic and preventative aspects of poisonous plants in livestock	Logan, Utah	Yes	3-F
ADP a4	<u>Diseases and parasites of horses</u>			
ADP a4-2 (CA) **	Equine infectious anemia	Pullman, Wash. College Station, Tex. Baton Rouge, La. Ames, Iowa	Yes Yes Yes No	4-B 4-B 4-B
ADP a4-3 **	Swamp fever in equines			

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			Summary of Progress	Area & Sub- heading
<u>ADP a5</u>	<u>Investigations of infectious and non-infectious diseases of poultry</u>			
ADP a5-21	Airsacculitis in turkeys	Ames, Iowa St. Paul, Minn. Madison, Wisc.	No Yes Yes	5-A 5-A 5-A
ADP a5-23	Infectious bronchitis in poultry	Ames, Iowa	No	
ADP a5-28	Newcastle disease	Ames, Iowa Orano, Maine Madison, Wisc.	No Yes Yes	5-D 5-D 5-D
ADP a5-29	Investigation of <u>Mycoplasma</u> infections in chickens	Athens, Ga. Raleigh, N.C. Univ. Georgia, Athens, Ga.	Yes Yes Yes	5-A 5-A 5-A
ADP a5-30	<u>Salmonella</u> infections in chickens	Athens, Ga.	Yes	5-A
ADP a5-31 (CA)	Investigations of blue comb in turkeys	St. Paul, Minn. Madison, Wisc. College Station, Tex.	Yes Yes Yes	5-G 5-G 5-G
ADP a5-32 (CA)	Investigations on Arizona (paracolon) infections in turkeys	St. Paul, Minn.	Yes	5-H
ADP a5-33 (CA)	Investigations of <u>Salmonella</u> infections in turkeys	St. Paul, Minn.	Yes	5-B
ADP a5-34 (CA)**	Investigations on hemorrhagic enteritis in turkeys	Blacksburg, Va.	No	
ADP a5-35	Viral respiratory diseases of chickens related to condemnations	Athens, Ga.	Yes	5-D
ADP a5-36	Investigation of <u>Mycoplasma</u> infections in chickens	State College, Miss.	Yes	5-A
ADP a5-37	Susceptibility of chickens to respiratory infections in controlled environments	Athens, Ga.	Yes	5-E
<u>ADP a6</u>	<u>Infectious and noninfectious diseases of fur animals</u>			
ADP a6-7 (R)	Field and laboratory studies of diseases of fur animals	Pullman, Wash. Madison, Wisc.	Yes Yes	6-A 6-A
ADP a6-9 **	Diseases of domestic rabbits	Fayetteville, Ark.	Yes	6-B
ADP P-1	Persistence and transmission of viral and rickettsial diseases in helminths	Pullman, Wash.	Yes	6-C
<u>ADP a7</u>	<u>Miscellaneous infectious and noninfectious diseases of animals</u>			
ADP a7-14 (R)	Fractionation, purification, and characterization of the components of normal and immune serums of animals	Ames, Iowa	Yes	7-A
ADP a7-16 (R)	Preparedness for laboratory assistance in diagnosis of foreign animal diseases	Greenport, N.Y.	Yes	7-B
ADP a7-17 (R)*	Studies to develop alleviators and diagnostic tests for plant poisoning and methods to avoid harmful residues in animal tissues from ingesting chemically treated plants	Logan, Utah	Yes	3-F
ADP a7-18 (R)	Investigations in livestock of the biochemical effects of agricultural chemicals and control substances	Kerrville, Tex. Nacogdoches, Tex.	Yes Yes	7-C 7-C
ADP a7-19 (R)	Detoxication mechanisms in cattle and sheep	Kerrville, Tex.	Yes	7-D

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			Summary of Progress	Area & Sub- heading
ADP a7-20 (R)	Characterization of cytological responses to toxic actions of pesticides and other agricultural chemicals in livestock and poultry	Kerrville, Tex.	Yes	7-E
ADP a7-22*	Studies of the incidence and pathology of cancer and other tumors in food-producing animals	Ames, Iowa	Yes	7-F
ADP a7-23 (R)	Toxicological and pathological effects of insecticides, herbicides, fungicides, and other agricultural chemicals on livestock and poultry	Kerrville, Tex. College Station, Tex.	Yes	7-G
ADP a7-24	Mycotic diseases of domestic animals	Ames, Iowa	Yes	7-H
ADP a7-25	Pasteurellosis disease in livestock and poultry	Ames, Iowa	Yes	5-C & 7-I
ADP a7-28	Proteins and other complex molecules from animal disease agents derived primarily from surface structures and extra-cellular products	Ames, Iowa	No	
ADP a7-29	Chemical and physical studies on microbial antigens	Ames, Iowa	Yes	7-I
ADP a7-30	Microbiology of the ruminant digestive tract and its relation to digestive disturbances	Ames, Iowa	Yes	7-J
ADP a7-31	Physiology of normal mammalian cells grown in tissue cultures	Ames, Iowa	No	
ADP a7-32	Metabolic, antigenic, and pathogenic characteristics of <u>Dermatophilus congolensis</u>	Ames, Iowa	Yes	7-K
ADP a7-33	Delineation of motor centers in the brain that are associated with motility of the ruminant esophagus and stomach	Ames, Iowa	Yes	7-L
ADP a7-34	Physiological fate of rumen gases absorbed from the lungs following eructation	Ames, Iowa	Yes	7-M
ADP a7-35	Correlation of the ultrastructural and biological properties of animal pathogens	Ames, Iowa	Yes	7-N
ADP a7-36	The effects of mycotoxins on animals	Ames, Iowa	No	
ADP a7-37	Relationship between psittacosis-group agents found in wild and domestic birds and domestic mammals	Ames, Iowa	Yes	5-F
ADP a7-38	Teratogenic and toxic compounds from poison plants	Logan, Utah	Yes	7-O
ADP a7-39	The role of parathyroid hormone and thyrocalcitonin in calcium metabolism	Ames, Iowa	Yes	7-P
ADP a7-40	Studies of pituitary-adrenal function in cattle	Ames, Iowa	Yes	7-Q
ADP a7-41	The toxicological effects of loco plants on livestock	Logan, Utah	Yes	7-R
ADP a7-42	Development and modification of equipment for greater laboratory and animal room safety	Ames, Iowa	Yes	7-S
ADP a7-43	The role of physical, chemical, and biological aerosols in domestic animal diseases	Ames, Iowa	Yes	7-T
ADP a7-44 **	Teratological effects of carbamate pesticides in domestic animals	College Station, Tex.	Yes	7-U
ADP a7-45 **	Effect of nitrates and other nitrogenous compounds on the toxicity of pesticides to livestock	College Station, Tex.	Yes	7-V
ADP a7-46 **	Cellular reaction to intracellular microbiological agents	Ames, Iowa	Yes	7-W

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			Summary of Progress	Area & Sub- heading
ADP a7-47**	Neurological effects of pesticides in domestic animals	College Station, Tex.	Yes	7-X
ADP a7-48**	Preparedness for laboratory assistance in diagnosis of duck virus enteritis (duck plague)	Greenport, N.Y.	Yes	7-Y
ADP a7-49**	Toxicological effects of herbicidal-treated plants on livestock	Logan, Utah	Yes	7-Z
ADP a8	<u>Foot-and-mouth and other exotic diseases of cattle</u>			
ADP a8-8 (R)	Immunological investigations - Studies on foot-and-mouth disease virus	Greenport, N.Y.	No	
ADP a8-10 (R)	Immunological investigations to determine the mechanism of antibody formation using viruses of exotic animal diseases	Greenport, N.Y.	No	
ADP a8-12 (R)	Development of methods for production of large quantities of foot-and-mouth disease virus by tissue culture methods	Greenport, N.Y.	No	
ADP a8-14 (R)	Establishment and characterization of cell lines and cell strains for the propagation of foot-and-mouth and other exotic disease agents of cattle	Greenport, N.Y.	No	
ADP a8-17 (R)	Mechanism of the interaction between foot-and-mouth disease virus molecules and other exotic viruses with their host cells	Greenport, N.Y.	Yes	8-A
ADP a8-18 (R)	Investigations of the genetic biochemistry of foot-and-mouth disease virus	Greenport, N.Y.	Yes	8-B
ADP a8-19 (R)	Effects of certain chemical and physical environments on foot-and-mouth disease virus	Greenport, N.Y.	No	
ADP a8-20 (R)	Bulk freeze-drying of foot-and-mouth disease virus, vaccines, and antiserums	Greenport, N.Y.	No	
ADP a8-25	Identification, purification, and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses	Greenport, N.Y.	Yes	8-C
ADP a8-26	Immuno-chemical investigations of foot-and-mouth disease	Greenport, N.Y.	Yes	8-D
ADP a8-27	Microbiological investigations - Attenuation of representative types of foot-and-mouth disease virus	Greenport, N.Y.	No	
ADP a8-29 (R)	Studies on the biological mechanisms of natural resistance and susceptibility of foot-and-mouth disease virus	Greenport, N.Y.	No	
ADP a8-30	Biological alterations of foot-and-mouth disease virus from continued residence in cell cultures	Greenport, N.Y.	Yes	8-E
ADP a8-31	Morphologic aspects of virus-cell relationships	Greenport, N.Y.	No	
ADP a8-32	Diagnostic and immunizing procedures for contagious bovine pleuropneumonia	Greenport, N.Y.	Yes	8-F
ADP a8-33 **	Carriers of foot-and-mouth disease virus	Greenport, N.Y.	No	
ADP a9	<u>Foot-and-mouth and other exotic diseases of swine</u>			
ADP a9-1 (R)	Immunological investigations of foot-and-mouth disease of swine	Greenport, N.Y.	Yes	9-A
ADP a9-2 (R)	Investigations of African swine fever	Greenport, N.Y. Kenya, East Africa	Yes No	9-B

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			Summary of Progress	Area & Sub- heading
<u>ADP all</u>	<u>Foot-and-mouth and other exotic diseases of sheep</u>			
ADP all-1	Immunological investigations of foot-and-mouth disease of sheep	Greenport, N.Y.	No	
<u>ADP b1</u>	<u>Parasites and parasitic diseases of cattle</u>			
ADP b1-23 (R)	Host-parasite relationship of coccidial parasites of cattle	Auburn, Ala.	No	
ADP b1-25 (R)	Clinical and physiological aspects of roundworm parasitism in cattle including anthelmintic action	Davis, Calif.	Yes	11-A
ADP b1-26 *	Investigations of trichomonad parasites	Logan, Utah	Yes	11-B
ADP b1-27 *	Host-parasite relationship of intestinal worms, <u>Cooperia</u> species, in cattle	Auburn, Ala.	Yes	11-C
ADP b1-28	Epizootiological-ecological investigations of the internal parasites of grazing cattle	Beltsville, Md.	No	
ADP b1-29 *	Etiology and immune response of cattle to winter coccidiosis	Logan, Utah	Yes	11-D
ADP b1-30	Anaplasmosis of cattle	Beltsville, Md.	Yes	11-E
ADP b1-31 *	Interrelationship of diet and parasitic infection in the production of cattle	Auburn, Ala.	No	
ADP b1-33	Parasites of cattle, with emphasis on <u>Stephanofilaria</u> species	University Park, N.M.	Yes	11-F
ADP b1-34 *	Effect of stocking rate and rotational grazing on internal parasitism of cattle	Auburn, Ala.	No	
ADP b1-35	Effect of host diet on the bionomics of the preparasitic stages of nematodes in cattle feces	Experiment, Ga.	No	
ADP b1-36	Life history and host-parasite relationships of <u>Trichostrongylus affinis</u> , a nematode parasite of rabbits	Auburn, Ala.	Yes	11-G
ADP b1-37	Effects of level, rate, and period of exposure to larvae on the establishment and pathogenesis of gastrointestinal nematode parasites of cattle	Experiment, Ga.	Yes	11-G
ADP b1-38 **	Pathogenesis of gastrointestinal nematodiasis in cattle	Beltsville, Md.	No	
ADP b1-39 **	Interrelationships of the level of parasitism and stocking rate of beef yearlings on winter temporary pastures	Beltsville, Md.	Yes	11-H
ADP b1-40 **	Cytochemistry of the enzymes of <u>Eimeria stiedae</u> , the cause of hepatic coccidiosis of the domestic rabbit	Auburn, Ala.	No	
ADP b1-41 **	Chromosome studies of various species of coccidia and nematodes parasitic in ruminants	Auburn, Ala.	No	
ADP b1-42 **	Oxygen uptake of coccidia and nematodes parasitic in ruminants and laboratory animals	Auburn, Ala.	No	
<u>ADP b2</u>	<u>Parasites and parasitic diseases of swine</u>			
ADP b2-12 (R)	Investigations of the swine intestinal roundworm, <u>Ascaris sum</u>	Lincoln, Neb.	Yes	12-A
ADP b2-15 (R)	Investigations of strains of <u>Trichinella spiralis</u> resistant to heat and cold and modes of transmission of the parasite	Beltsville, Md.	Yes	12-B

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			Summary of Progress	Area & Sub- heading
ADP b2-17	Studies of <u>Strongyloides ransomi</u> infections in baby pigs	Tifton, Ga.	Yes	12-C
ADP b2-18	Evaluation of biochemical and other aspects of the host-parasite relationship in the development and severity of helminthiasis of swine	Beltsville, Md.	No	
ADP b2-19	Life cycle of the short-tail nodular worm of swine, <u>Oesophagostomum brevicaudum</u>	Tifton, Ga.	No	
ADP b2-20	Factors involved in the infection of the dung beetle, <u>Phanaeus vindex</u> , with the larvae of the thick stomach worms of swine	Tifton, Ga.	Yes	12-D
ADP b2-21 (CA)	Biology and control of <u>Stephanurus dentatus</u> , the swine kidneyworm	Raleigh, N.C.	Yes	12-E
ADP b3	<u>Parasites and parasitic diseases of sheep and goats</u>			
ADP b3-16	Gastrointestinal nematodes and nematodiasis of sheep and measures for their control	Beltsville, Md. Lexington, Ky.	Yes Yes	13-A 13-A
ADP b3-19	Studies on the life cycles of <u>Eimeria ahsata</u> and <u>E. crandallis</u> , pathogenic coccidia of sheep	Auburn, Ala.	Yes	13-B
ADP b3-20	The effect of gastrointestinal nematodes on the tensile strength and sulfur content of wool	Fargo, N.D.	Yes	13-C
ADP b3-22 *	Control of the common sheep scab mite, <u>Psoroptes ovis</u>	Albuquerque, N.M.	Yes	13-F
ADP b3-23	Chemical control of <u>Oestrus ovis</u> in sheep	Albuquerque, N.M.	Yes	13-D
ADP b3-24	Biology and control of <u>Psorergates ovis</u> , the Australian itch mite of sheep	Albuquerque, N.M.	Yes	13-E
ADP b3-25	Pathobiology of several laboratory and field strains of <u>Psoroptes ovis</u> , the mite of common sheep scab	Albuquerque, N.M.	Yes	13-F
ADP b3-26 *	Overwinter survival of parasitic nematode larvae on Mississippi pastures	Auburn, Ala.	No	
ADP b3-27	The biology and control of the liver fluke, <u>Fasciola hepatica</u> , in the southwest	Las Cruces, N.M.	Yes	13-G
ADP b3-28 **	The biology and control of worm parasites of sheep in the southwest with emphasis on immunization procedures for the control of haemonchosis	Las Cruces, N.M.	Yes	13-H
ADP b4	<u>Parasites and parasitic diseases of poultry</u>			
ADP b4-9*	Investigations for controlling coccidiosis of poultry	Beltsville, Md.	No	
ADP b4-10*	The biology of the nematode parasite of poultry and related birds with special reference to the application of findings to control measures	Beltsville, Md.	No	
ADP b4-11	Biological investigations of protozoan parasites and parasitic diseases of poultry	Beltsville, Md.	Yes	14-A
	Blackhead of turkeys	College Station, Tex.	Yes	14-A

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			Summary of Progress	Area & Sub- heading
ADP b5	<u>Treatments for removal or control of parasites of domestic animals</u>			
ADP b5-18	Investigations to develop new and improved chemical agents for the treatment, prevention, or control of helminthic parasites in farm animals	Beltsville, Md.	Yes	15-A
ADP b5-19	Potential chemotherapeutic agents as treatments for bovine venereal trichomoniasis	Beltsville, Md.	Yes	15-B
ADP b5-20	Evaluation, development, and standardization of antiparasitics of established, or reported value	Beltsville, Md. Experiment, Ga.	Yes Yes	15-C 15-C
ADP b5-21	Control of lice on cattle	Albuquerque, N.M.	No	
ADP b6	<u>Miscellaneous parasites and parasitic diseases</u>			
ADP b6-9 (R)	Publication of author, subject (parasite) and host index-catalogues of medical and veterinary zoology	Beltsville, Md.	Yes	16-A
ADP b6-10 (R)	Investigation of immunologic and other biologic approaches to the prevention and control of parasitic diseases	Beltsville, Md. St. Paul, Minn. Madison, Wisc.	Yes Yes Yes	16-B 16-B 16-B
ADP b6-11	Studies of the chemical and physical elements of parasites and parasite-host relationships in animals	Beltsville, Md.	Yes	16-C
ADP b6-12	Taxonomic investigations of helminths and other parasites	Beltsville, Md.	Yes	16-D
ADP b6-13 (C)	Investigations on the serological diagnosis, transmission, and control of equine piroplasmiasis	Beltsville, Md. Gainesville, Fla. Lexington, Ky.	Yes Yes Yes	4-A 4-A 4-A
ADP b6-14	Maintenance of author, parasite-subject, host, and anthelmintic catalogues and checklist of specific and subspecific names	Beltsville, Md.	Yes	16-A
ADP b6-15	Maintenance of parasite collections	Beltsville, Md.	Yes	16-E
ADP b6-16	Identification of parasites of importance in parasitological research, regulatory, and quarantine, and other work	Beltsville, Md.	Yes	16-D
ADP b6-17	Pigments of parasites	Beltsville, Md.	Yes	16-F
ADP b6-18	Biology, epidemiology, and pathogenicity of demodectic mange of domestic livestock	Albuquerque, N.M.	Yes	16-G
ADP b6-19	Cytological investigation of protozoan parasites that penetrate the gastrointestinal tract of poultry and other farm animals	Beltsville, Md.	Yes	16-H

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			Summary of Progress	Area & Sub- heading
	<u>P. L. 480 Projects</u>			
A6-ADP-1	Leucocytozoon infection in chickens and development of effective treatment	Taipei, Taiwan, China	Yes	14-A
A7-ADP-5	Vaccine for protecting sheep against sheep pox	Madras, India	Yes	10-A
A7-ADP-23 **	Jaagziekte and maedi (pulmonary adenomatosis complex) of sheep and goats	Mathura, U. P., India	Yes	3-H
A7-ADP-24 **	Lymphosarcoma of bovines with particular reference to Indian buffaloes	Izatnagar, U. P., India	No	
A7-ADP-29 **	Studies on salmonella infections in domestic animals and birds to provide information on the natural reservoirs and mode of transmission of infections from animal to animal and animal to man	Hissar, Punjab, India	No	
A10-ADP-5 *	Pathogenesis of lesions produced by the local leech, <u>Limnatis nilotica</u> of Israel	Jerusalem, Israel	No	
A10-ADP-6 *	The immunizing effect of <u>Brucella</u> cell wall	Jerusalem, Israel	Yes	1-K
A10-ADP-7	Lipid metabolism of the animal disease parasites, <u>Trypanosoma congolense</u> and <u>Trypanosoma vivax</u>	Jerusalem, Israel	No	
A10-ADP-8	Effects of prolonged feeding of terephthalic acid to rats	Jerusalem, Israel	Yes	7-BB
A10-ADP-9	The structure, chemical composition, immunochemistry and nutritional requirements of PPLO (<u>Mycoplasma</u>) pathogenic to farm animals	Jerusalem, Israel	Yes	5-A
A10-ADP-13 **	Genetic and other biological and immunological properties of foot-and-mouth disease virus	Jerusalem, Israel	No	
A22-ADP-4	<u>Gastrophilus pseudo-hemorrhoidalis</u> (equine parasite)	Ankara, Turkey	Yes	4-C
A22-ADP-6	Preparation of a vaccine against sheep pox from tissue culture propagated virus	Ankara, Turkey	Yes	10-A
A22-ADP-7 *	Study of the horsesickness virus	Ankara, Turkey	Yes	4-D
A22-ADP-8	Studies of various indigenous types of foot-and-mouth disease virus, and the production of a vaccine for the control of FMD in Turkey	Etlik, Turkey	Yes	8-H
A22-ADP-9	Experimental neurosurgical problems	Ankara, Turkey	Yes	7-CC
E21-ADP-6 *	The immunogenic properties, virulence, and tropism of Newcastle disease virus strains adapted to immune serum	Pulawy, Poland	No	
E21-ADP-7 *	Environmental stress as a contributory factor in animal disease	Pulawy, Poland	No	
E21-ADP-9	Trichinellosis with special reference to epizootiology, immunology, and pathogenesis	Wroclaw, Poland	Yes	12-B
E21-ADP-12 **	Immunological response of sheep and goats to strongyloidiasis	Warsaw, Poland	Yes	13-I
E21-ADP-13 **	The changeability of biological properties of viruses and the interrelationship between hosts and viruses (Newcastle disease virus)	Pulawy, Poland	No	
E25-ADP-3	Decarboxylating enzymes of plant and animal origin	Madrid, Spain	No	
E25-ADP-4 *	Diagnostic methods for African swine fever	Madrid, Spain	Yes	9-B

Line Project Check List - Reporting Year July 1, 1966 to June 30, 1967

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Incl. in	
			Summary of Progress	Area & Sub- heading
E29-ADP-4	Etiological factors of scrapie in sheep	Edinburgh, Scotland	Yes	3-G
E29-ADP-5	Investigation of scrapie, a transmissible disease of sheep of obscure etiology	Compton, England	Yes	3-G
E30-ADP-1 **	Respiratory diseases in young cattle	Belgrade, Yugoslavia	No	
E30-ADP-2 **	Studies on the immune response to infection with the liver fluke, <u>Fasciola hepatica</u>	Beograd-Zemun, Yugoslavia	No	
S3-ADP-2 *	Studies on foot-and-mouth disease	Sao Paulo, Brazil	Yes	8-G
S3-ADP-3 *	Plants of the state of Sao Paulo poisonous to domestic animals	Sao Paulo, Brazil	Yes	7-AA
S8-ADP-1 *	Environmental factors influencing parasites and parasitic diseases of economical importance in ruminants (cattle, sheep, alpacas)	Lima, Peru	Yes	11-I
S9-ADP-1 *	Anaplasmosis, piroplasmosis, and babesiel- losis in cattle	Montevideo, Uruguay	No	
<p>* - Discontinued during reporting year ** - Initiated during reporting year</p>				

